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PHYSIOLOGICAL REQUIREMENTS OF ROCKY MOUNTAIN TREES[1]

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I. INTRODUCTION

The most casual observer ascending any mountain range in the western United States can hardly fail to be impressed with the fact that there is a sharp line of demarcation between the grassy plains at the base of the mountain and the wooded slopes of the mountain proper. As one ascends farther, gradual or sometimes very abrupt changes are noted in the character of the forest cover. With increase in altitude the forest generally becomes more dense, trees of greater stature are observed, and those who are able to distinguish note the occurrence of new species in each altitudinal zone. To a lesser degree the same differences in the forest cover may be noted on opposing slopes at the same elevation—that is, slopes facing the south bear forests similar to those at lower elevation, while those facing the north have the character of higher altitudes. The vegetation of ridges is always different from that of ravines at the same altitudes.

The laymen will recognize that these differences in the forest cover are the result of different "growing conditions" at different elevations, just as a person who had lived in the South would recognize, almost instinctively, that the growing conditions of the mountain valleys of Colorado could not possibly be suited to the cultivation of corn or cotton.

While the intensive study of the relations of plants to the soil and climatic conditions which comprise their environment goes under the formidable name "ecology," ecological knowledge is not confined to scientists and, in fact, has been common property for ages. The writer has had opportunity to observe the first impressions of a great many people who were visiting the western mountains for the first time, and has been impressed by the amount of logic exhibited in relating cause and effect in the matter of forest distribution. Why does this southerly exposure bear an open, scrubby forest of yellow pine, and that northerly exposure, directly opposite, bear a much more dense stand of vigorously growing firs? Often the people who know no botany and much less ecology take in the situation at a glance, at least so far as it is possible to do so from superficial evidence.

To the forester such questions are of the utmost practical importance. Not only is it the forester's business to know the trees with which he

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[2] This project has been under the direction of the writer since its inception in 1910, and he assumes full responsibility for the quality of the work done and for the conclusions deduced from the data. It is, however, a pleasure to acknowledge the great efforts which have been required of a number of observers in the accumulation of the records. The records furnished by the Weather Bureau are duly acknowledged, and the efforts of all those numerous and changing observers who have created these records. The records of the Wagon Wheel Gap and Fremont Experiment Stations are the result of the concerted effort of many regular observers, all of whom deserve credit. The original installation of instruments at the Wagon Wheel Gap Experiment Station was made, and the records were obtained for two years, under the direction of B. C. Kadel, of the Weather Bureau.

is daily working, their relative and absolute demands for moisture, light, heat, and soil fertility, but he must be ready to make practical use of such knowledge in formulating his plans for reforestation and in deciding upon the amount and kind of timber which may safely be cut from a given area. Consciously or unconsciously he is daily making use of whatever knowledge he may possess as to the physiological requirements of each species in his region—that is, those properties of the tree which determine that it will grow best under certain conditions of soil and atmosphere. It is of the utmost importance that this knowledge should not be superficial and that through the increase in scientific facts forestry should be removed from its empiric basis.

Unfortunately, much of our knowledge of tree physiology and tree requirements is still very vague, and it is the aim of forest ecology to increase, systematize, and analyze such knowledge. As a means of systematizing the knowledge of trees, in this report we speak of an area of forest of essentially uniform composition as a forest type; and it is assumed, since the composition of the forest is uniform over a given area, that the environmental conditions which have brought this uniform forest into existence must be just about the same over the whole area, or at least must have been the same at the time when the forest started. Usually a forest type is given the name of the tree species which predominates and gives it its essential character, even though a great many other species may occur in the same stand in lesser numbers. Thus a forest in which Douglas fir is the most prominent tree, with occasional neighbors of spruce and pine, would be spoken of as a "Douglas fir type." The word "type" is somewhat loosely used to refer to the ground occupied as well as to the forest itself.

A broader use of the word "type" is as a synonym for forest zone or altitudinal zone. It is true that the character and composition of the forest changes gradually with a change in altitude, and, for example, most of the ground between elevations of 8,000 and 10,000 feet, in a given region, might be occupied by Douglas fir stands. But, from the lower extremity, a great many strips of yellow pine forest might extend into this zone on the warmer, southerly exposed areas, and likewise from the upper edge there would extend belts of spruce. As a general term, therefore, the word "zone" is far preferable to "type," and the latter will be used in this report only for specific areas bearing forests of uniform character.

As a further distinction between forest areas, the forester has brought into use the word "site" to describe the producing qualities of the ground with respect to any particular species. Thus among many Douglas fir types, all of which were characterized by the predominance of Douglas fir over other species, it might be desirable to distinguish those of the best quality as "Douglas fir sites I" and those of very meager producing qualities as "Douglas fir sites III." Such distinctions are usually based upon the evidence of the tree growth itself—that is, either the apparent rate of growth determined, for example, by the general vigor of the stand, or the growth rate actually measured, is the best possible evidence of the quality of the ground. But there is every reason to believe that the productive capacity of the ground can be measured, sooner or later, in terms of the soil quality and the atmospheric conditions which simultaneously affect tree growth, so that any systematic effort to study forest types and to describe those qualities which distinguish them must inevitably be at the same time a study of sites.

OBJECTS OF THE PRESENT STUDY

Having now shown in a general way the nature of the problem which is constantly under consideration by students of ecology and forestry, the purpose of the study (one phase of which is covered by the present paper) may be definitely stated. As a matter of fact, it has several purposes, which are by no means distinct, but worthy of individual enumeration.

1. To compare the environments of the several species of trees with respect to each condition which may be separately measured, in order to determine what particular conditions have the most important bearing on the initiation of new stands, favoring one species rather than another.

2. After noting the differences in the conditions of the various types, which really indicate differences in the physiological functioning of the species, to determine experimentally the degree of such differences as may exist between the species, and, as a result, the degree of difference in the actual requirements of the species for optimum growing conditions. This second object can hardly be attained in the field without most extensive long-term study, and necessarily resolves itself into experiments under controlled conditions of temperature, light, moisture, etc. The present paper deals largely with the results of such experiments.

3. To describe the conditions of the various forest types of the region in such a manner as will explain most clearly the reasons for the success or failure of artificial forestation, so far as these may result from the environment, and the conditions and means necessary for successful natural regeneration after fires or cutting. Here the object is to lead away from empiric silvicultural "systems" and toward the attainment of definite environmental conditions in all silvicultural practice.

4. To convey a conception of the conditions under which the Rocky Mountain forests exist—that is, a view of the climatic and soil conditions of the forest region as a whole.

It is needless to state that not all of these objects have been attained in the present work, which should be considered as something of a pioneer effort, merely blazing the way for much greater efforts and more refined methods which are necessarily for the future to bring forth. The hope may be justified, however, that the results and conclusions of this study will add somewhat to the information on the general subject and encourage the doing of more intensive work.

SCOPE OF WORK

The entire work, of which this paper represents only a part, is mainly a study of the physical environments of a number of different forest types in Colorado and Wyoming. The detailed study of the composition of these several types has not been given a great deal of attention. Since the forest conditions are common ones and are frequently encountered in the region, it is believed that the indications of prevailing composition and of changes in composition, as they may be expressed in general terms, will be as valuable for present purposes as tabulated statements of the number, size, and kind of trees found at the several stations. The observations of meteorological conditions and of certain soil conditions thought to be fully as important, have, therefore, comprised the major part of the field work.

However, the early efforts (*1*) [3] to summarize and compare these site conditions, as given in meteorological and soil records, and to deduce

[3] Reference is made by number (italic) to "Literature cited," p. 163–164.

therefrom the full reasons for the presence or absence of a given species on the various sites were so far from satisfactory that the need for controlled experiments to indicate the comparative reactions of the several species has long been apparent. The amount accomplished in this line is not insignificant, and, since the results aid very materially in interpreting the field conditions, we have chosen to present, first, all of the physiological data that are available. In other words, we shall first consider the manner in which the several species are affected by more or less controlled conditions in the laboratory or greenhouse; then, perhaps, we may weigh more precisely the importance of each condition encountered in the field, and especially avoid the almost universal and inevitable error of placing reliance upon the measurement of a condition which has not really been measured or expressed in the proper terms.

Of the meteorological records which are used in this report the larger share have been obtained by the Forest Service at the Fremont Experiment Station, near Manitou, Colo., and by the Weather Bureau and Forest Service, cooperating in an intensive project at the Wagon Wheel Gap Experiment Station, near Wagon Wheel Gap, Colo. It has been the aim to conduct the meteorological work at both these stations on a high plane of accuracy, with equipment as complete as was thought necessary. At both places the observations have covered elevations from the station headquarters at about 9,000 feet to timber line at 11,000 or 12,000 feet. In both cases, too, soil moisture and soil temperature data have been secured during most of each period of atmospheric observations.

In addition, the Forest Service has equipped and operated from the Fremont Station three subsidiary stations at which the Weather Bureau had previously started the usual observations of precipitation and air temperature. These stations were manned by forest officers, and a very diligent effort has been made to obtain continuous records for them. The stations in question are at the Monument Nursery, in the yellow pine type near Monument, Colo., at the Foxpark Ranger Station, lodgepole pine type, at Foxpark, Wyo., and in the Nebraska sand hills, where the planting of yellow pine and jack pine has reached such large and successful proportions. The first station is only about 20 miles north of the Fremont Station and for all practical purposes may be considered as belonging in the Pikes Peak altitudinal series.

Although the Weather Bureau records of precipitation and air temperature, secured through cooperative observers, were available for a large number of stations in the region covered by this report, and many such stations are within the mountain forest zone, only limited use has been made of such records, and for the following reasons:

(*a*) Many such observation points are situated, with the small towns, in deep valleys where the conditions met with, especially those of air drainage and soil composition, are not at all the conditions of the adjacent forested slopes. In fact, nearly all such valleys, especially if they possess a distinct flood plain, are devoid of forest cover, and to use their weather records in this study might lead to very erroneous conceptions though the temperature conditions alone are probably not responsible for the absence of forests.

(*b*) The records obtained at all such mountain stations are only those of precipitation and air temperature. No soil data whatever are secured.

The special work in this project dates from January 1, 1910, when the control station at Fremont was equipped. Records obtained up to 1918

have, in general, been employed, the summarizing of data having been started at that time. However, some records particularly needed have been added since 1918. The period of operation, as well as the equipment of the several other stations, is given under the respective descriptions.

METHOD OF STUDY

As has already been indicated, the primary data to be presented in this study are records of climatic and soil conditions in different forest types, and the main object of such presentations must be to show that differences in climatic and soil conditions, between the forest types, either do or do not exist in sufficient degree to account for the varying phenomena of occurrence and growth.

Since the special data collected by the Forest Service in no case cover periods long enough to establish the "normal" conditions of any of the forest types (and by this we mean the average conditions for a period of at least 10 years), and since even "normal" conditions as established by 10 years of observation are certain always to be changed by the addition of further records, it is necessary that we adopt short-period records as a basis for comparison. Such adoption can not be fraught with any serious dangers when the forest types to be compared are in the same general locality, that is, in the path of the same air currents, storm centers, etc. Any considerable separation of the stations, however, especially in a rugged mountainous region, is likely to introduce temporary variations in certain conditions which are not "normal," and particularly in those factors which are most directly influenced by the paths of storm centers. Thus, at the moment of writing this statement, it appears that the storm centers have for some time been passing considerably to the north of the Pikes Peak region, giving that locality rather unusual westerly winds and leaving it with a dearth of moisture, so that at the end of May an unprecedented shortage of water exists. Scarcely 100 miles to the north, unusually large accumulations of snow are reported at the same moment. Again, the moisture factor is most variably influenced by the restricted character of many of the summer showers; especially are the heaviest downpours in a given locality likely to affect only a very small area.

Temperatures are generally not so directly affected by local conditions. Thus the month of December, 1917, was not only an unusually warm month at the Fremont Station but showed the same character over a large part of the western United States, and January, 1918, was, likewise, generally cold to an unusual degree.

We may, therefore, feel safe in comparing the records of any two near-by stations for short periods, whatever the factor under consideration, and we shall demand an increasing period as the distance between stations increases.

Fortunately, the dozen or more stations located in the vicinity of Fremont all come under the same general influences. This is true of the entire area from the plains to the summit of Pikes Peak, with the exception that summer rains frequently fall in one part of the area without wetting other parts. Winter snows may also be so localized, but usually in conformity with altitudinal zones. It is true, two stations not 100 yards apart may on a given day have temperatures varying by a couple of degrees in one direction and on the next day varying in the

opposite direction. Such variations from a consistent relation are, however, always small, and there is every reason to believe that the means of a single month usually express essentially the normal temperature relation between two stations for that month of any year.

Therefore the method of study and the method of presenting results is that of comparing each factor for any station with the corresponding factor at the control station for whatever period observations may have been taken at the outlying station. As a basis for this comparison we have the record of each factor at the control station, measured with practically no variation in method from January, 1910, to date. Exception should be made here to the measurement of evaporation, in which a satisfactory method was not attained until 1917.

The detailed methods of taking meteorological observations, so far as they vary from the standard methods of climatologists the world over, will be described in connection with each condition measured.

REVIEW OF OTHER WORK ALONG SIMILAR LINES

Although much systematic ecological study has been attempted in the United States and other countries, and the western portion of our own country has offered an especially attractive field for ecologists because of the sharp contrasts in vegetation and causative factors which are found in relatively small areas, still the main field with which foresters are concerned has barely been scratched. Several studies, which might have been very productive, scarcely satisfy the forester's requirements because of the lack of long-term records.

Be that as it may, a number of authors have obtained facts and deduced conclusions as to the distribution of our Central Rocky Mountain forest trees which we can not afford to overlook. No attempt will be made at this stage to introduce these facts, which may better be mentioned in connection with my own discussions and conclusions. I shall merely list here the works which have a direct bearing on the major problems, and with no attempt to cover the general or specific physiological studies.

Clements (*7*, *8*, *10*) in three of his works presents many valuable ideas regarding the relation of Rocky Mountain vegetation to environmental conditions, and with particular reference to the Pikes Peak region, in which much of his investigation has been conducted. The latest of Clements's books, "Plant Succession" (*10*), published in 1916, may be said to cover the entire ground of the earlier works, bringing all of his observations under one comprehensive theme, namely, the changes which occur in the character of the vegetation of a given area as the result of reactions of the plant forms upon the environment and of gradual changes in the climatic and edaphic (soil) conditions.

In a more specific work Clements (*9*) gives to foresters a much more concrete idea of the requirements of an important tree species, lodgepole pine, with lesser data on its common associates.

Ramaley (*16*, *17*), working near Boulder, Colo., and in the deeper mountains at Boulder Park, has likewise made numerous observations on the forest types and zones of Colorado. His papers, however, make no claim of extensive systematic measurement of physical factors and hence can be considered as having only suggestive value in connection with the present work. In both of the papers cited the theme is a classi-

fication of Colorado forests according to moisture conditions and composition.

Shreve (*24*, *25*), working at the Desert Laboratory at Tucson, Ariz., and in the Santa Catalina Mountains adjacent thereto, has in a number of papers published results directly bearing on the subject in hand. "The Vegetation of a Desert Mountain Range" is the most comprehensive of these papers and covers most fully the ultimate problems, beyond pure climatology, with which the forester is concerned. Although the Santa Catalinas are somewhat different from the Central Rockies in being surrounded by desert on nearly all sides and in having a different seasonal distribution of rainfall, yet it is apparent that the limiting factors for the occurrence of a given species must be essentially the same in the two regions; else all attempts to formulate a systematic ecology would be vain. These factors may not, it is true, appear quantatively alike under present methods of measurement, but, if so, we should seriously question the method of measurement.

It will be noted that in the papers referred to Shreve ascribes the main control of the upward extension of desert plants to temperature, and in another paper (*23*) he has quite convincingly shown that the duration of freezing temperatures is all-important with plants accustomed to the ordinarily warm winter air of the desert. With this view we shall have no reason to take issue. The other main conclusion, that lack of moisture limits the downward extension of the forest species, individually and collectively, will, it is believed, be found subject to question or at least modification.

Robbins (*18*) has prepared the most recent and complete summary of Colorado's climatic conditions in relation to native vegetation and agriculture. While, as stated by the writer, this work attempts to show only a qualitative relation between climate and plants, it is, nevertheless, excellent both in the data systematically presented and in the relations described. For the most part these relations are too broadly stated to be of direct assistance in the present study. A quotation from the discussion of the freezing of plants is of considerable technical interest:

> It is a familiar observation that some of the more tender plants are injured by temperatures above the freezing point; and that, on the other hand, there are many plants that may withstand temperatures considerably below the freezing point. This statement may apply not only to dormant plant parts, but to swelling buds, open flowers, and forming fruit as well. The plants at timber line and above are subject to freezing temperatures almost every night in the year. The exact nature of this immunity to low temperatures is not known.

Weaver (*27*) in 1917 studied the desert-to-mountain formations of Washington in a manner not unlike Shreve's, and ascribes the changes in vegetation mainly to increasing soil moisture and decreasing evaporation with a rise in elevation.

Shantz (*20*, *21*) has dealt with problems intimately connected with the factors limiting the downward extension of the Rocky Mountain forests. Particularly is Shantz's work enlightening to foresters in the thorough treatment of the soils problem. He has made it plain that the lighter soils of the plains, characterized by bunch grass, show much less variation in productivity from year to year than the heavier, loamy soils which develop the grama-buffalo-grass association. This difference is due to greater penetration of both moisture and roots in the lighter soil as well as to the greater availability of the moisture when the content becomes low, tending to encourage slow growth, and the longer lived bunch grass.

By analogy we may say that the same relation exists between grassland or sage brush and the lowest type of forest, commonly called "woodland" by foresters, since it is obvious that there can be no important change in climatic conditions in the small space between the centers of development, and that the development of the forest is made possible by the slight soil changes resulting from elevation, surface erosion, and leaching, all of which maintain a younger soil.

The establishment of the forest experiment stations in the western United States, beginning with that at Flagstaff, Ariz., in 1908, gave unparalled opportunity for the collection of forest and climatological data over a period of years. As a result, studies similar to the present one have been initiated in Arizona, California, and Idaho, and the study of forest and herbaceous vegetation has been carried on in connection with a number of experiments at the Utah Station, where grazing problems occupy the attention first. The strictly forest studies, however, are for the most part not yet ready for publication.

Pearson (*14*) at the Flagstaff Station early investigated the effect of yellow pine forests upon local climatic conditions, by securing data in the forest, the edge of the forest, and "parks" (grassy, meadowlike openings) of considerable extent. While the climatic conditions recorded by Pearson are interestingly compared with our own, this study can not be said to throw very much light on the conditions governing different forest types.

However, Pearson (*15*) has recently made available the results of observations at a series of stations in the San Francisco Mountains, in a very comprehensive way, and we shall have reason frequently to compare his conditions with our own.

We have, similarly, had access to an unpublished report by Larsen [4] on the conditions of Montana and Idaho, which has been extremely helpful in giving comparable data.

The problem of the prairies in the Middle West, and their physical relation to the occasional forested areas, has received considerable attention, and this problem is not too remote from our own entirely to lack interest. On this subject may be considered the work of Shimek (*22*), who concludes, regarding Iowa conditions:

> 1. Exposure to evaporation as determined by temperature, wind, and topography is the primary cause of the treelessness of the prairies.

and

> 3. Rainfall and drainage, while of importance because determining the available supply of water in both soil and air, are not a general, determining cause, both frequently being equal on contiguous forested and prairie areas.

Shimek also dismisses fires as a cause of the absence of forests. It is believed that the later conclusions of Weaver and Thiel (*26*, *28*), with reference to Minnesota, are essentially in agreement with this. The point which seems to have been overlooked here, and in all similar discussions, is that forests occur usually on the slopes of ravines or on hillsides, where the old soil is being rejuvenated by a secondary erosion and where, even with less moisture than in the heaviest soils, the availability may be greater. Considered from this angle, the occurrence of forests in the prairie region is exactly parallel to their occurrence on the first mountain elevations at the edge of the plains.

[4] LARSEN, J. A. CLIMATIC STUDY OF FOREST TYPES, DISTRICT 1. U. S. Dept. Agr. Forest Serv., unpublished report, 1918.

It will be fairly evident from the reading of the few treatises which have been mentioned that the region under discussion has not been neglected by ecologists. It will be equally evident that there is still room for much systematic effort in the study of the environmental factors in order that the theories advanced regarding the distribution of mountain forests may be more thoroughly tested by well-established facts. The most apparent fact, after considering all of these regional studies, is that so far ecology has given the physics of the soil-moisture problem entirely inadequate attention.

II. PHYSIOLOGICAL STUDIES LEADING TO AN INTERPRETATION OF THE ENVIRONMENTAL DATA

It is quite generally recognized that the result of studying any condition in nature, even when the method of study is strictly quantitative, should not consist wholly in presenting the accumulated facts but quite as much in placing a logical interpetation on those facts. In the present study we are dealing not only with a great variety of natural conditions which require quantitative expression but with a variety of growing entities whose behavior and reaction to known conditions can not be determined by casual observation. For example, the mere fact of finding a spruce tree growing at the water's edge does not prove that the tree uses or requires an unusual amount of water, much less that it is growing in that particular spot primarily because of the moisture, or even indirectly because of the moisture. It would be as logical to say that because the aligator spends a good deal of his time in the water, he must drink and must require for physiological processes an extraordinary amount of water. This may not be true at all; he may be a most abstemious animal.

The point is that in ecology we dare not take the conditions of growth as prima facie evidence of the requirements of growth, even though it be true that none of the conditions can be altered without affecting the character of the growth. This is especially true when we are compelled, as in the present instance and in most ecological studies so far made, to speak of requirements in a relative rather than an absolute sense; that is, when we are simply trying to compare the requirements of several species rather than determine them absolutely for any species. This may be illustrated by a point which has appeared very forcefully in the present study. Taking the superficial appearance of soil conditions as a measure of relative requirements, foresters have repeatedly stated that the moisture requirements of spruce were greater than those of yellow pine. Now, there could be no objection to saying, and probably no error in saying, that spruce requires or at least develops best in a fresh, moist soil of high water-holding capacity. This would be an absolute expression which would simply gain in accuracy as the soil conditions were further analyzed. We might infer, and would be likely to do so, because of the character of the soil occupied, that spruce must use a great deal of water in its development. Such an inference would be unwarranted, but would be especially dangerous if we should say, comparatively, that spruce uses more water than pine. Here we are treading on absolutely unsafe ground. On the face of it there is no scientific basis for such a statement, if we use simply the evidence of the field conditions observed. And even if this were true of the spruce forest in the aggregate, that bespeaks nothing as to the individual.

It has been, therefore, in the hope of partially overcoming the inherent weakness of a comparative field study that certain observations have been made under laboratory conditions, permitting a better knowledge of the trees themselves, hence a safer interpretation of the environmental conditions which surround them in the field, and, perhaps, a clearer conception of how those conditions should be measured in the future to express a logical relation between the environment and growth.

For the most part these special observations have been made upon the four important species which are involved in the field study, namely, western yellow pine, Douglas fir, lodgepole pine, and Engelmann spruce. Two other species, forest "weeds," have been studied to some extent, namely, limber and bristlecone pines (*Pinus flexilix* and *P. aristata*). A few observations have also been made on the Lake States pines and other conifers not indigenous to the Rocky Mountains.

In the interest of brevity, some details of the conditions of these experiments may have been omitted which might be considered as having important bearings. Anyone wishing to investigate these details will be given all possible assistance.

TRANSPIRATION TESTS IN 1917

To establish the water requirements of some of the Rocky Mountain trees in the same terms as used by Briggs and Shantz (*6*) for agricultural crops, and to determine the relative transpiration rates of the species as a basis for gauging their moisture requirements in the field, transpiration tests were conducted in the greenhouse of the Fremont Experiment Station for a period of about six months in 1917. The experiment was repeated in 1920.

It should be recognized at the outset that the greenhouse did not present natural conditions for the growth of any of the species, the air temperatures being higher than commonly occur except possibly in the lowest zone of the region, and the air movement considerably less than the wind which would occur in any situation out of doors. For these reasons, though we may speak of the "absolute water requirements" of the trees in this particular test, these requirements are not an indication of what the water use might be under any other conditions; and it would be best, as Briggs and Shantz have done, to assume only that we have established relative requirements of the several species for one set of conditions. These relations may or may not hold good under other conditions. Briggs and Shantz found that relative water requirements of different species did not vary much under different conditions, though the absolute requirements of all might be twice as great during a dry season as during a moister one. Thom and Holtz (*26*) found that the physical conditions might vary sufficiently to change even the relative requirements of different species, but their more important result was to show that the absolute water requirement increases with the availability of moisture.

It would appear that the high temperatures and low wind velocities occurring during these tests should tend to stimulate assimilation rather more than transpiration, so that the absolute water requirements here would be less than under normal field conditions for any one of the species. This, however, may not be true. For this and other reasons appearing later it is difficult to compare the absolute requirements with those of agricultural crops.

MATERIAL STUDIED

As the means were not at hand for treating large trees in the intensive manner required in such a study, efforts were confined to nursery specimens, 5 and 6 years old at the outset. These had all been developed in the nursery of the Fremont Station, with practically uniform soil conditions and with no artificial watering except as small seedlings, so that all should have been in much the same condition at the outset.

Two specimens each of yellow pine, Douglas fir, lodgepole pine, Engelmann spruce, limber pine, and bristlecone pine were taken for potting, while a third specimen of each was taken at the same time for drying, in order that the initial dry weight of the specimens to be grown might be computed. This drying and all other dryings required in these studies were done in hot water bath or controlled hot air oven at a temperature of about 92° C. and without vacuo.

To determine the initial green weights, and also the green weights at the close of the test period, each tree was washed to remove adhering soil particles, whipped vigorously through the air to remove free water, and placed immediately on the scales. After this the potting was accomplished as soon as possible.

The initial weights varied from 7 to 14 gm. and heights from 3 to 6 inches, the spruce being, on the whole, best developed for its age. No measurements other than weights were taken at the outset. At the end of the test period the green weights were taken; each tree was photographed to scale, as shown in Plates 1 to 3; measurements were made to determine the mean needle dimensions of each tree and the ratio of surface to volume (the whole volume having been determined by immersing the top in water to the root collar); finally the remains were oven dried, and later the dry material was reduced to ash in a porcelain dish over a Bunsen flame.

From the volume displacement and needle dimensions we are enabled to compute the area of leaf surface in each case, with a very considerable but general error on account of the stem volume included. This will, at least, give some basis for comparison with other experiments in which the leaf surface is the basis for calculations of water loss. Because of the great inaccuracies involved in the method and the practical impossibility of applying it to a large tree, and also because it is believed that transpiration is so largely controlled by the area exposed to insolation and the consequent total absorption of radiant energy, we have also used another basis for expressing leaf area, which we shall call "leaf exposure." This is obtained from the tree photographs, which are against a background of cross-section paper, by estimating the proportion of each square inch which is obscured by the foliage. This method, if carefully followed, gives reasonably consistent results, except in cases like tree No. 8, in which the focus is bad.

It is seen that the "leaf exposure" could not be more than one-third of the whole leaf surface, and owing to a great deal of overlapping of needles, as well as elimination of stem, the data in this case compare generally on a basis of about 1 to 6. But with the limber and bristlecone pines, whose foliage is very compact, the ratio is more nearly 1 to 10.

SOIL

For potting, open-topped galvanized cans were used, 4 inches in diameter and 10 inches deep. No drainage openings were made in the

cans; but, to encourage aeration of the soil, a 2-inch florist's pot was inverted in the bottom of each can, and a glass tube one-eighth inch in diameter was so bent and placed that its lower end opened into the pot and its upper end just above the rim of the can. This tube served for supplying the necessary water and was at all times left open for aeration of the soil. It is believed that the amount of vaporization through the tubes was insignificant, though no control tests were made at the time. We are enabled to approximate the loss from this source by the observations in 1920. However, in 1917, the soils were never allowed to become greatly heated, the potting cans being placed in similar cans having diameters of 6 inches, so that the sun never shone on the lower portions of the pots.

Before weighing and potting, each tree was trimmed so that the longest roots would not be cramped in the can. The longer roots were spread around the porous pot in the bottom of the can, and the others were placed as the pots were filled, so as to be evenly distributed throughout the soil. When the cans were all filled to within a half inch of the tops, they were sealed with a 2 to 1 mixture of paraffin and vaseline, which held very well throughout the season in spite of occasional melting.

The soil used was a specially prepared loamy sand of granitic origin, containing considerable humus derived mostly from leaves of limber pine and *Arctostaphylos* sp. Both sand and humus were sifted through one-eighth-inch screen. The resultant mixture was what would ordinarily be considered a good potting soil. It was thought to be desirable to insure an abundance of nutrient material, and there is no reason for supposing that this was overdone.

None of the soil placed in the pots was oven-dried, but a weighed amount of air-dried soil was used in each, and during the process of potting several samples were taken for the purpose of determining the moisture content. The net oven-dried weight for each pot was then computed.

The saturation capacity of the soil used was originally determined to be about 40 per cent, and, in accordance with Kiesselbach's (*12*) finding that transpiration occurs most freely when the soil is about half saturated and the theory of Hilgard (*11*) that half saturation permits the desired aeration, 20 per cent moisture was adopted as the standard at which the soil would be kept. Later it was found that with greater compactness this saturation might be much less, and after centrifuging, as low as 25.8 per cent. However, the figure 31.9 probably applies most nearly to the condition of the soil in the pots. The corresponding capillarity was 28.2 per cent, and the moisture equivalent at 100 gravity was 11.05 per cent, using the term in the same sense as it is used by Briggs and Shantz (*5*) for the water-holding capacity under a force of 1000 gravity. The mean wilting coefficient was determined in 1920 to be 3.47 per cent for Douglas fir and 3.91 per cent for spruce, or an average value of 3.69 per cent. On this basis, and assuming that the 20 per cent maximum moisture was evenly distributed, we should have as the availability $\frac{20.-3.69}{20}=0.816$. It is more probable that the moisture within reach of the roots, at the bottom of each pot, was 25 per cent or more, making the availability at least 0.850.

Table I shows all of the fundamental data regarding the trees, the amounts of soil used, and the gross weight of the pots as they were maintained throughout the season.

TABLE I.—*Description and condition of trees used in transpiration measurements*

TABLE I.—*Description and condition of trees used in transpiration measurements*

Species	Yellow pine.		Douglas fir.		Lodgepole pine.		Engelmann spruce.		Limber pine.		Bristlecone pine.	
Can No.	1	2	3	4	5	6	7	8	9	10	11	12
Age of trees (years)	5	5	5	5	5	5	5	5	6	6	6	6
Initial green weight (gm.)	10.55	13.48	6.77	8.90	7.52	8.80	14.38	13.46	8.10	14.50	10.85	10.15
Green-dry factor of sample tree	2.45	2.45	2.56	2.56	2.51	2.51	2.32	2.32	2.37	2.37	2.41	2.41
Initial dry weight (computed)	4.31	5.50	2.65	3.48	3.00	3.51	6.20	5.80	3.42	6.12	4.50	4.21
Final weight:												
Green	16.97	20.56	10.05	16.50	13.28	15.20	26.06	28.50	12.15	16.82	15.39	14.26
Dry	5.56	7.46	4.28	5.85	4.32	5.24	10.41	10.58	4.48	6.47	5.95	5.49
Weight gain for season:												
Green	6.42	7.08	3.28	7.60	5.76	6.40	11.68	15.04	4.05	2.32	4.54	4.11
Dry	1.25	1.96	[a] 1.63	2.37	1.32	1.73	4.21	4.78	1.06	[b] .35	1.45	1.28
Ash:												
Weight (gm.)	.31	.30	.20	.49	.25	.34	.50	.64	.29	.33	.39	.35
Percentage of final green weight	1.83	1.46	1.99	2.97	1.88	2.24	1.92	2.25	2.39	1.96	2.53	2.45
Computed leaf surface:												
Volume of top by displacement (cc.)	8.8	11.9	7.4	10.3	7.0	8.7	21.1	19.1	8.5	9.3	9.7	8.8
Length of needles (cm.)	7.23	9.87	2.20	1.90	4.90	5.58	1.83	1.53	3.00	3.37	3.07	3.02
Cross section (mm.)[c]	1.13	1.30	1.83	1.20	1.12	.93	.90	.77	.92	.90	.80	.73
	×	×	×	×	×	×	×	×	×	×	×	×
	.65	.78	.42	.38	.53	.45	.70	.48	.78	.75	.75	.53
Ratio of area (in sq. mm.) to volume (in cu. mm.)	5.22	4.46	6.72	6.92	6.08	7.20	5.08	6.77	6.61	6.91	7.36	8.75
Computed area (sq. cm.)	459	531	497	713	426	626	1,072	1,293	562	643	714	770
Leaf exposures (sq. cm.), from photographs	64	79	64	100	56	66	153	149	57	66	71	71
Net dry-soil weight (gm.)	2,580	2,550	2,540	2,516	2,479	2,562	2,380	2,398	2,566	2,502	2,476	2,405
20 per cent water (gm.)	516	510	508	503	495	513	476	479	513	501	496	481
Tare (gm.)	1,022	980	996	1,001	973	988	1,037	1,012	1,015	977	1,017	1,009
Gross, with tree as above	4,128	4,054	4,051	4,029	3,955	4,072	3,907	3,902	4,102	3,994	4,000	3,905

[a] At end of tests, all root tips are old and lignified.
[b] Root tips very short, showing slow or late development.
[c] Cross sections of yellow pine and lodgepole pine computed as semicircles; limber pine and bristlecone pine as fifth circles, more or less; Douglas fir as elipses; Engelmann spruce as rectangles.

PROCEDURE

The trees were taken from the nursery and potted about April 15, 1917. From that time until April 26 they were kept in a warm room, without sunlight, to encourage root growth and establishment in the soil. On April 26 the cans were first brought to standard moisture content, and measurements of transpiration losses were begun. From that date to June 3 they were kept in the window of a warm room, where they received light for only a few hours each day. The pots were frequently but not regularly shifted in position.

On June 3 they were placed on a revolving table in the greenhouse, where they remained until the close of the test on November 14, with the exception of one day out of doors. This table was handled in several ways, the power available for rotating it at the outset being inadequate. At first a small motor was used, the motor being cut in each minute for a period of a second or more, so as to give the table a fraction of a revolution. For several short periods the table was turned by hand, a quarter revolution about once each hour. A water motor was finally used, which for a time kept the table revolving continuously. This, however, seemed to have a theoretically objectionable feature in that the trees were constantly passing from light to shadow, in a very unnatural manner. The driving belt was therefore arranged so as to move the table a perimetral distance of about 6 inches each minute, or, say, a complete revolution in about 25 minutes.

The pots occupied the periphery of the 4-foot table, various types of evaporimeters being placed between them. Within this circle was placed an air-and-soil thermograph, the arm of the air register being shaded, while the soil bulb was blackened and so placed, with its long axis horizontal, as to receive as much sunlight as the trees. With the assistance of maximum and minimum registering air thermometers and a thermometer attached to the blackened bulb of the soil thermograph, there were thus recorded both air temperatures and "sun" temperatures. In addition, a psychrometer was used during the morning observation each day, giving a rough indication of prevailing vapor pressures.

The most important question of procedure, of course, concerns the method of determining water losses. As shown by Table I, each pot had, at the outset, a known gross weight when its soil contained 20 per cent of moisture. The aim was to keep the moisture to this standard by replacing losses each day. It was only necessary to determine the amount of water required to bring the pot up to standard weight in order to record the loss for the preceding period. This was accomplished by placing the pot on one pan of the scale, the standard weights on the other pan, and filling from a titrating burette until a balance was reached. The amount drawn from the burette was, therefore, the measure of the loss. The measurements of transpiration, it is thus seen, were actually volumetric, even though scales were used. This introduced no error worth considering, as the temperatures at the observation hours varied scarcely at all from about 50° F.

The burette was graduated to 0.1 cc. The scales were barely sensitive to 0.1 gm. under the usual load of 4,000 gm. However, errors from this source should be compensating. Whenever the filling was carried too far, as not infrequently happened, the overload was determined and allowed for, and also carried to the record for the next period.

All observations were made in the early morning and before sunlight had reached the trees, and when, therefore, the transpiration rate would be almost at its minimum. The order of measurements was invariable, and the time rarely varied more than 15 minutes from the standard.

Now, in fact, though it has been stated that the plan was to maintain standard moisture in each can, it is readily seen that the moisture was most of the time below standard. The extent of the ordinary depressions was very small. The largest single loss between fillings, 157.1 gm., would mean a moisture content at the end of the period of 13.9 per cent, or a depression of 6.1 per cent. The average periodic loss of the heaviest water-user was 23.76 gm., and the average depression below standard moisture, therefore, only 0.93 per cent. This average depression would reduce the availibility of the moisture only from the approximated value 0.852 to 0.846.

On the other hand, the distribution of the moisture from top to bottom of each pot, as shown by examination at the end of the tests, was not all that might be desired. The lowest inch of soil was practically saturated, and above this the moisture decreased so that just below the paraffin the soil was only freshly moist. In spite of this, rootlets had penetrated to all sections of the soil. It seems evident that, except with the most extreme depletion noted, there was probably within reach of the longest roots at all times practically saturated soil.

The atmospheric conditions of the greenhouse, as has been stated, were not such as would occur naturally in any of the sites where these species grow. At times the ventilators were kept closed and the air temperatures were allowed to go as high as they would with full sunlight. At other times the ventilators were opened and all possible draft was developed; and, of course, under these conditions the air of the greenhouse did not become so warm. Again, sunlight was excluded on certain days to see what effect this would have on transpiration rates. On two or three occasions when cloudy weather prevailed, an interval of 2 or 3 days was allowed to elapse between measurements, since the losses were very small. On two occasions when the writer was not there to make the measurements, the intervals were considerably longer, the trees being shaded by canvas for the entire period.

No apparent injury resulted from the high temperatures in the greenhouse, except to tree No. 4, Douglas fir. On June 24, which was a clear day of exceptionally low humidity and high evaporation rate, two of the newly formed shoots on this tree wilted and did not recover. Nevertheless the tree continued to function properly. The other Douglas fir (No. 3) followed No. 4 very closely, but after the first of September showed a gradual descrease in its response to transpiration stimuli and when unpotted was found to be deficient in new root growth. In the normal trees it appeared that many of the root tips had continued growth to the end of the season, while in this one growth had evidently ceased much earlier.

Tree No. 10, limber pine, at the end of the season showed very short growing tips on the roots, indicating that root development had been very sluggish or had started very late. This sluggishness is doubtless related to the small weight accretion.

RESULTS

The amount of transpiration for each tree during each period of the study from April 26 to November 14 has been tabulated, and it is found that the tree-to-tree relations hold very closely, from day to day, in spite of great variations in the environmental conditions. It is not relevant to present purposes to present the detailed data. In Table II the transpiration by months is given and the tree performances are summarized. In correcting for the loss of water directly from the soil, the amount of 107 gm. for the season has been arrived at by considering the daily loss the same as in 1920. While in 1917 the pots were more fully protected from the sun, yet considerably higher air temperatures were attained, so that this allowance seems justified and can hardly be enough in error appreciably to affect the results.

TABLE II.—*Summary of transpiration in the test of 1917*

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Species	Yellow pine.		Douglas fir.		Lodgepole pine.		Engelmann spruce.		Limber pine.		Bristlecone pine.		All.
Tree No.	1	2	3	4	5	6	7	8	9	10	11	12	
Transpiration by periods:													
April and May—													
Grams	42.1	187.5	143.3	157.1	189.9	221.3	223.6	223.3	86.3	86.7	137.5	136.6	1,835.2
Per cent[a]	2.3	10.2	7.8	8.6	10.4	12.0	12.2	12.2	4.7	4.7	7.5	7.4	.....
June—													
Grams	360.8	704.2	337.2	348.8	329.7	411.0	433.5	535.1	265.7	215.2	322.4	388.5	4,652.1
Per cent	7.8	15.2	7.2	7.5	7.1	8.8	9.3	11.5	5.7	4.6	6.9	8.4	.....
July—													
Grams	285.1	427.9	221.5	235.3	182.9	208.9	232.5	354.0	173.5	132.5	175.8	213.6	2,843.5
Per cent	10.0	15.0	7.8	8.3	6.4	7.3	8.2	12.5	6.1	4.7	6.2	7.5	.....
August;—													
Grams	388.4	571.6	293.0	323.8	256.7	296.3	302.6	581.0	244.6	203.1	266.9	297.7	4,025.7
Per cent	9.7	14.2	7.3	8.0	6.4	7.4	7.5	14.4	6.1	5.0	6.6	7.4	.....
September—													
Grams	402.1	515.4	155.8	334.1	208.9	238.9	342.9	578.1	234.0	189.1	260.6	268.7	3,728.6
Per cent	10.7	13.8	4.2	9.0	5.6	6.4	9.2	15.5	6.3	5.1	7.0	7.2	.....
October—													
Grams	382.6	479.5	44.9	260.2	176.1	202.4	303.3	506.4	215.5	171.7	266.8	249.9	3,259.3
Per cent	11.8	14.7	1.4	8.0	5.4	6.2	9.3	15.5	6.6	5.3	8.2	7.6	.....
November—													
Grams	201.4	250.3	10.2	89.2	85.8	92.7	134.2	221.1	100.3	72.5	126.3	115.9	1,499.9
Per cent	13.4	16.7	.7	6.0	5.7	6.2	8.9	14.8	6.7	4.8	8.4	7.7	.....
Total, April to November (grams)	2,062.5	3,136.4	1,205.9	1,748.5	1,430.0	1,671.5	1,972.6	2,999.0	1,319.9	1,070.8	1,556.3	1,670.9	21,844.3
Correction for direct evaporation	107	107	107	107	107	107	107	107	107	107	107	107	1,284
Net loss through leaves	1,955.5	3,029.4	1,098.9	1,641.5	1,323.0	1,564.5	1,865.6	2,892.0	1,212.9	963.9	1,449.3	1,563.9	20,560.3
Transpiration:													
Grams per gram weight accretion—													
Green	305	428	335	216	230	244	160	192	300	415	319	381	263
Dry	1,565	1,545	675	693	1,002	905	444	606	1,144	2,750	999	1,221	880
Grams per gram mean weight	142	178	131	129	127	130	92	138	100	62	110	128	123
Grams per square centimeter leaf surface	4.26	5.7	2.21	2.3	3.11	2.5	1.74	2.24	2.16	1.5	2.03	2.03	2.48
Grams per square centimeter leaf exposure	30.6	38.4	17.2	16.4	23.6	23.7	12.2	19.4	21.3	14.6	20.4	22.0	20.6

[a] Percentage of the transpiration of all the trees.

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Water Requirements

This term, as used by Briggs and Shantz (*6*), denotes the units of water used by a plant for the production of a unit of dry plant material.

The first computation in the lower section of Table II gives corresponding data for the small trees whose transpiration has been measured. Averaging the results for each pair of specimens, we find the species arranging themselves according to Table III. In this table the use per unit of green weight is also given, since the green-weight accretions were directly measured, whereas the original dry weights were obtained indirectly, as explained.

TABLE III.—*Water requirements*[a]

Species.	Water used per unit of—	
	Dry-weight accretion.	Green-weight accretion.
	Gm.	*Gm.*
Limber pine	1,947 (678)	358 (49)
Yellow pine	1,555 (8)	366 (52)
Bristlecone pine	1,110 (94)	350 (26)
Lodgepole	954 (41)	237 (6)
Douglas fir	684 (8)	276 (50)
Engelmann spruce	525 (68)	176 (14)

[a] The probable error in the average of two figures is indicated by the quantity in parentheses.

Considering only the first column of figures, it is seen that the probable error in the averages is large in two or three cases, and especially so with limber pine, so that this species might possibly belong after yellow pine in the list. In fact, considering that it is the specimen of high water requirement (Tree 10, 2,750) which showed at the end of the season evidence of lack of vigor, it seems altogether probable that the normal or true water requirement of limber pine should be gauged by the lower figure. Also, from the fact that No. 10 was at the beginning a larger, probably more succulent specimen, we may quite confidently place this species on a par with bristlecone pine.

No other change in the order of arrangement is indicated as probable by the variations in the first column. However, examining the second column of the table, it is seen that the requirement of Douglas fir is greater than that of lodgepole. But, again, it is the Douglas fir specimen of higher water use (No. 3, 335) which behaved abnormally, its activity apparently almost ceasing before the end of the season, so that we must incline toward leaving Douglas fir in the position indicated by the first column of figures.

We shall not attempt here to discuss the cause of these variations, though that, too, is most interesting and will be at least partially clarified later.

It must be recognized that the relative water requirements, or ability to make growth with a given volume of water, while having a direct bearing on the relations of two or more species which compete with each other, may tell very little as to the ability of a tree of a given species to withstand the drought or wind exposure of a given site. The water requirements no doubt explain in some degree the gradual suppression

and crowding out of yellow and limber pines by fir or spruce, the similar elimination of lodgepole when spruce seriously competes with it. The water requirements may explain certain things which we have habitually ascribed to presence of or lack of shade "tolerance." But it does not necessarily mean that yellow pine, for example, might not resist transpiration and survive under rather rigorous conditions where no question of relative growth rate was involved.

Resistance to Transpiration

To obtain a better idea of relative resistance to transpiration we should consider the water losses, under equal conditions, as related to plant mass, leaf area, or leaf exposure. In Table IV the data are summarized on each of these bases, but the species are arranged in the order indicated by the relative transpiration per unit of leaf exposure.

TABLE IV.—*Water losses per unit of leaf area and plant mass*

Species.	Seasonal water loss (grams).		
	Per square centimeter of leaf.		Per gram mean green weight.
	Surface.	Exposure.	
Yellow pine	4.98	34.5 (3.)	160
Lodgepole	2.80	23.6 (0)	128
Bristlecone	2.03	21.2 (0.7)	119
Limber pine	1.83	18.0 (2.8)	81
Douglas fir	2.26	16.8 (0.3)	130
Englemann spruce	1.99	15.8 (3.0)	115

On the basis of the transpiration per unit of leaf exposure (which is believed to be the safest basis we have) or per unit of mass, the order of arrangement is essentially the same as in Table III. It is, perhaps, significant that the four important forest trees, yellow pine, lodgepole, Douglas fir, and spruce, appear in the same order as in the preceding table, while limber pine and bristlecone pine have moved to positions just below lodgepole. Taking the data at face value, let us consider for a moment what these qualities of limber and bristlecone pine must mean. In the first place, it has been seen that these species, which are admittedly very adaptive "weed" trees, use considerable water without making much growth. In the second place, we see that relative to their leaf area or whole mass they use very little. In other words, they are in some way adapted to protect themselves from water loss, but along with that adaptation, perhaps as a result of it, they have very meager ability for making use of light in photosynthesis. It is readily seen how this may happen. If either of these trees, accustomed to growing on bare sites where there is no competition, has adapted itself through reduction in the number and size of its stomata, moisture loss is reduced and the ingress of carbon dioxid is likewise reduced. If, again, as the writer has sometimes noted, the needles are closely appressed for the purpose of mutual protection, then moisture is saved at the expense of the full insolation of each leaf. The same might be the result of thickened epidermis or heavy palisade tissue. It thus appears that almost any adaptation for the conservation

of water must result in inefficiency in photosynthesis, and it might possibly be stated as an axiom that a weed is a plant which through protective adaptations is facultative for a variety of environmental conditions but which for the same reasons is incapable of making a standard rate of growth.

The impression is, then, obtained that yellow pine, lodgepole, Douglas fir, and spruce are resistant to transpiration in almost the same degree as they are capable of making use of water for their development, and that none of them possess any special adaptations for preventing water losses which interfere with growth. The cause for the differences between the several species should, then, be sought in those internal conditions which may determine their photosynthetic capacities and the behaviors of their cell contents as solutions, as was done by Salmon and Fleming (*19*) in the study of the winter hardiness of grains.

Before turning to that subject, however, which will be considered under the heading "Sap density," the transpiration rates should be further analyzed.

Periodic Transpiration

In Table II the transpiration of the trees by months has been shown, with the amount for each tree expressed as a percentage of that for all the trees. From these data it may be observed that only a few of the trees maintained stable positions with respect to the whole. The greatest significance of this is to indicate that, if the growth could have been measured for shorter periods, the relative water requirements might not have been the same as those for the whole season.

Comparing the transpiration at the beginning and end of the season (April to June against October to November), it is found that the species may be arranged in the following order, those which show the greatest relative increases being placed first: Yellow pine, limber pine, spruce, bristlecone, Douglas fir, and lodgepole. Lodgepole and Douglas fir show actual decreases.

If we should eliminate trees 3 and 10, which were apparently affected by some unknown factor, it would scarcely change the relations of the species.

These data at least indicate that the internal conditions which control transpiration are variable and probably are affected by the building of new tissue, accumulation and distribution of carbohydrates, and other changes which may occur in a season's growth.

Response of Transpiration to Light and Air Movement

The variations of each tree during the season, as shown above, almost preclude the possibility of determining the responses of the species to the different environmental conditions which were produced from day to day, since such comparisons, to use the available data, must include days during all parts of the season.

Taking as a standard for each species the days during the season when there was no ventilation in the greenhouse and when the total recorded sunlight was in excess of 400 minutes per day, it is found, as shown by Table V.

1. That for days having from almost none to 400 minutes of sunlight, with other conditions equal, all the species show about 60 per cent of the transpiration for a standard day.

2. With bright diffuse light, such as might penetrate the canvas curtains on a sunny day, the transpiration varies from 21 per cent of standard for lodgepole to 32 per cent for yellow pine and Douglas fir.

3. With dull diffuse light, as on cloudy days, the percentages vary from 17 per cent to 23 per cent of standard. Though lodgepole shows at all stages the greatest depression from the lack of sunlight, it is hardly safe to say that this is a specific character.

TABLE V.—*Response of various species to different conditions of light and air movement*

Conditions.	Number of days.	Species.					
		Yellow pine.	Douglas fir.	Lodgepole.	Engelmann.	Limber pine.	Bristlecone pine.
Average transpiration (cubic centimeters per day, standard), no ventilation, over 400 minutes sunshine	12	21.82	[a] 13.59	11.86	19.84	9.62	13.22
Proportionate transpiration:							
No ventilation, less than 400 minutes sunshine	11	.62	.62	.54	.62	.62	.59
No ventilation, bright diffuse light	19	.32	.32	.21	.26	.31	.25
No ventilation, dull diffuse light	7	.23	.20	.17	.22	.18	.18
Some ventilation, over 600 minutes sunshine, temperature over 75° F.	4	1.05	1.08	1.35	.98	1.18	1.18
Some ventilation, over 600 minutes sunshine, temperature under 75° F.	2	.91	1.02	1.12	.86	1.11	1.10
Some ventilation, 400 to 600 minutes sunshine, temperature over 75° F.	7	1.27	1.12	1.40	1.08	1.41	1.23
Some ventilation, 400 to 600 minutes sunshine, temperature under 75° F.	13	.99	1.06	1.19	.99	.94	1.02
Some ventilation, less than 400 minutes sunshine, temperature over 70° F.	10	1.00	1.05	1.06	.90	1.00	.99
Some ventilation, less than 400 minutes sunshine, temperature under 70° F.	8	.59	.58	.64	.61	.63	.57
Some ventilation, diffuse light	5	.21	.28	.28	.31	.28	.29

[a] Only tree No. 3 used, account seasonal change in No. 3.

4. With ventilation, the transpiration of all species is increased over that without ventilation, other conditions being about the same. The amount of ventilation in the greenhouse was not sufficient to produce striking changes. The exposure of the trees outdoors for one day did not materially increase the transpiration rate, temperatures being considered.

Finally, since the specific responses are subject to the seasonal changes already noted, we may examine the results expressed by the total transpiration of the 12 trees during each day or longer period.

An attempt has been made to relate this to the vapor deficit, or differential between the atmospheric vapor pressure and the saturation pressure conceived to exist within the leaf, as determined by mean temperatures. For the latter there is available either the air thermograph record or the "sun" thermograph (blackened bulb) record. The latter seems preferable in theory, but the record is not very trustworthy because of very large corrections in the instrument as used during most of the season. This, together with the fact that atmospheric vapor pressures were determined only in the early part of each period (9 a. m.) and at its end (7 a. m.), makes the computation of vapor deficits the roughest approximation. In addition, it becomes evident that the leaf temperatures (and saturation pressures) should not be considered as equal to the black-bulb temperatures, but more nearly equal to what the wet bulb of the psychrometer would show synchronously. As the psychrometric data are not sufficient even to approximate the mean wet-bulb temperatures, the results of "sun temperature" computations alone will be shown. Recognizing that transpiration at night, because of the lack of sunlight, is in a different category from that during the day and

should be given much less weight in the total, the general scale of temperatures has been raised by computing the daily means as follows: The 12 hourly temperatures from 7 a. m. to 6 p. m., inclusive, are added to the temperatures at 8 p. m., 12 m. and 4 a. m., and the total is divided by 15.

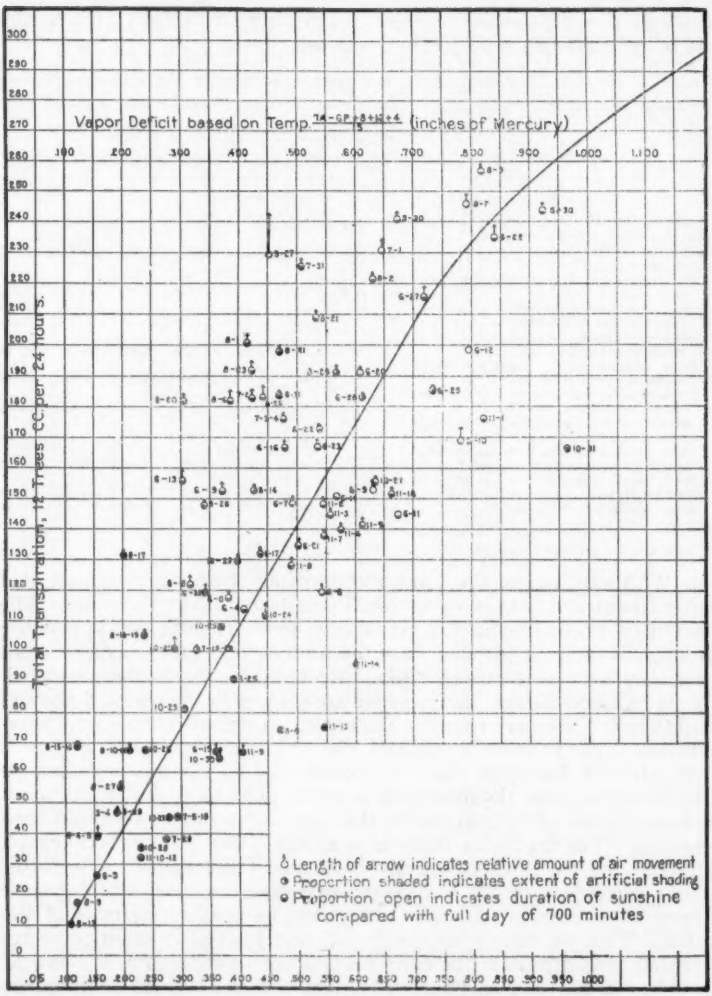
Vapor Deficit based on Temp. $\frac{T4-6P+8+12+4}{15}$ (inches of Mercury)
Total Transpiration, 12 Trees C.C. per 24 hours.
Length of arrow indicates relative amount of air movement
Proportion shaded indicates extent of artificial shading
Proportion open indicates duration of sunshine compared with full day of 700 minutes

FIG. 1.—Transpiration in relation to saturation deficits in 1917. Numbers opposite circles give month and day of observation.

In figure 1 the results of such computations of vapor deficit for 84 periods during the season of operations are plotted with the daily transpiration sums. The basic data will not be tabulated, since it is voluminous and has no precise value. Instead, the general character of each period is indicated by symbols on the diagram. The mean curve which may be obtained from the 84 points is only suggestive in showing—

1. That for a given saturation deficit a wide variation is possible in the amount of transpiration induced. It is believed, however, that this is largely the result of insufficient data for computing both saturation pressure in the leaf and atmospheric vapor pressure.

2. Air movement increases transpiration somewhat more than has been allowed by the computations, which, in fact, made no allowance for this factor except as it might influence the depression of the psychrometer wet bulb.

3. The amount of sunlight is not the cause of much variation in the results. If anything, the use of black-bulb temperatures has made a little too much allowance for sunlight effects; or, in other words, the plants are not quite so strongly affected by light as is the black-bulb temperature.

4. The transpiration rate (average) is quite proportionate to vapor deficits until the former approaches a daily amount equal to the total weight of the plants, when transpiration, apparently, does not quite keep up with saturation deficits.

5. The transpiration at the end of the season is less than in the earlier months, days of like valuations being compared. The size of the plants, of course, increased during the season, but the increase in leaf area was almost wholly during the first month. It seems safe to say that old leaf tissues do not permit as much transpiration as young tissues.

6. The physical control of transpiration and the lack of plant control is fairly evident, though by no means proved.

TRANSPIRATION IN 1920

The striking differences between the species, indicated by the transpiration rates in 1917, both on the basis of growth accretion and relative leaf areas or leaf exposures, called, first, for a reasonable explanation of the temporary circumstances which produced such results, and, secondly, for a repetition to determine whether similar relations of the species might hold with new and different material and whether either the absolute or relative water requirements might be different under more natural environmental conditions.

The first need had been definitely pursued in the interim, and the second was fulfilled during the summer of 1920. In repeating the transpiration tests, it was especially sought to have as large an assortment of plant material as could be adequately dealt with.

MATERIAL STUDIED

Twenty-three pots of plants were used, compared with the 12 in 1917. Of these one containing a large yellow pine failed early in the season. An additional pot without trees served to measure the possible direct water loss from the soil. In a number of cases, two or three specimens were handled as one in a single pot, in order to give a better average result. (See Plates 4 to 6.)

The limber and bristlecone pines and a single large spruce specimen were from the same lots of stock which were drawn upon in 1917, having been in the Fremont Station nursery during the interval. The remaining trees were all younger stock grown in the same nursery, and mainly of fairly definite origin as to seed. Where possible a variety of seed sources was represented in the selections for each species, in order to determine the possibilities of variation between geographic forms of the same species.

The data on the trees used are given in Table VI.

TABLE VI.—*Source, size, growth, and condition of trees in 1920 transpiration test*

Species and source of seed.	Age.	Number potted.	Green weight.	Similar specimens dried.		Calculated dry weight of potted trees.	Weight at end of season.				Seasonal weight gain.		Leaf exposure from photograph.	Pot No.	Remarks.
				Number.	Dry-green ratio.		Green.			Total dry.	Green.	Dry.			
							Living plant.	Air-dry salvage.	Total.						
Yellow pine:	*Years.*		*Gm.*			*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Sq. cm.*		
Harney, S. Dak.	4	2	21.46	2	0.394	8.46	21.29	0.95	22.24	9.45	0.78	0.99	86	22	Slow in beginning vernation.
Do.	4	2	18.84	2	.394	7.43	22.19	.37	22.56	8.88	3.72	1.45	85	1	
Bitterroot, Mont.	4	2	15.34	2	.400	6.14	20.64	.22	20.86	7.80	5.52	1.66	107	18	Do.
Tusayan, Ariz.	4	2	17.78	2	.377	6.70	27.40	.42	27.82	9.66	10.04	2.96	114	15	
Douglas fir:															
Hayden, Wyo.	8	1	28.20	2	.419	11.81	28.44	.84	29.28	13.60	1.08	1.79	144	17	Almost no small fibrous roots, but some new growth made.
Leadville, Colo.	8	1	34.65	1	.408	14.13	31.86	2.22	34.08	15.55	−.57	1.42	140	10	Large proportion of new foliage.
Pike, Colo.	8	1	21.30	2	.426	9.08	23.17	.20	23.37	9.80	2.07	.72	142	7	
Lodgepole pine:															
Colorado, Colo.	5	2	19.81	2	.409	8.10	28.16	.20	28.36	9.91	8.55	1.81	111	3	
Washakie, Wyo.	5	2	22.27	2	.405	9.02	32.90	.46	33.36	11.84	11.09	2.82	119	23	
Gunnison, Colo.	5	2	32.61	2	.390	12.72	45.69	.32	46.01	16.53	13.40	3.81	170	24	
Engelmann spruce:															
San Isabel, Colo.	4	3	11.89	7	.392	4.66	20.28	.32	20.60	7.33	8.71	2.67	103	9	
Montezuma, Colo.	4	2	5.38	6	.391	2.10	10.30	.10	10.40	3.99	5.02	1.89	68	19	
San Isabel, Colo.	4	3	7.27	2	.388	2.82	13.02	.12	13.14	4.90	5.87	2.08	84	12	
Unknown	8	1	55.69	1	.434	24.60	76.15	.77	76.92	30.64	21.23	6.04	295	11	Much slower than small spruces in beginning vernation.
Limber pine:															
Unknown	9	1	64.18	2	.448	28.76	73.36	.68	74.04	30.93	9.86	2.17	239	16	
Do.	9	1	32.47	2	.448	14.55	31.13	.28	31.41	14.64	−1.06	.09	119	21	Buds barely extended during season; roots grew reasonably well.
Bristlecone pine:															
Unknown	9	1	29.80	1	.444	13.23	31.59	.22	31.81	14.69	2.01	1.46	124	8	
Do.	9	1	12.55	1	.444	5.58	15.12	.06	15.18	6.60	2.63	1.02	70	4	
Scotch pine:															
Riga variety	4	1	29.70	1	.394	11.70	31.30	.26	31.56	13.62	1.86	1.91	155	13	
Do.	4	1	21.40	1	.394	8.44	27.50	.63	28.13	11.21	6.73	2.77	108	2	Slow in beginning vernation.
Siberian larch:															
Russia	6	1	9.87	2	.298	2.94	12.90	.98	13.88	5.87	4.01	2.93	98	6	Nearly all of foliage fallen and dry when final weight was determined; No. 6 dried earlier than No. 20.
Do.	6	1	15.38	2	.298	4.58	16.51	1.42	17.93	7.66	2.55	3.08	133	20	
All.	.....	34	527.84	47	400	.....	640.90	12.04	.....	.....	125.10	47.54	.....	.....	

SOIL

The soil in which the trees were potted was nearly the same as that used in 1917. There had been added to the original soil a somewhat more clayey sand of granitic origin, so that in 1920 we find a greater water-holding capacity, a slightly lower moisture equivalent, and a third higher wilting coefficient. The data on the 1920 soil are:

	Per cent.
Saturation	36.46
Capillarity	31.02
Moisture equivalent	10.42
Wilting coefficient (average for spruce and yellow pine)	4.88

It was planned to carry about 12 per cent moisture, and to prevent the saturation of the lowest stratum of the soil by injecting the daily supply of water near the surface. The feed tube, then, instead of opening into the inverted clay pot at the bottom of the can, was bent about 2 inches below the surface and opened into the soil near the center of the can. Aside from this the potting arrangement was the same as in 1917.

The 12 per cent moisture would constitute, in the average of the 22 cases, 284.4 gm. of water. At the outset, however, the average pot was given 528.8 gm. of water, in order to create a very favorable condition. In the average case this supply lasted much longer than had been anticipated and was not brought down to standard until after July 1, so that during the first two months the moisture conditions were by no means uniform.

Finally, beginning September 3, the water content was gradually reduced, until on September 27 it was 70 gm. below the standard, or in the average case amounted to 9.05 per cent. This change was designed to simulate the usual autumn drying of the soil.

We thus have the following average conditions in the several pots, comparing with a mean availability of about 0.850 through the entire season of 1917:

May 11, 528.8 gm. = 22.31 per cent = availability 0.780; decreasing to 284.4 gm., or 12 per cent, about July 1 = availability 0.594; reaching September 27, 214.4 gm., or 9.05 per cent = availability 0.461.

It is, therefore, seen that in the osmotic sense the conditions for ready absorption of the water were far less favorable in 1920 than in 1917.

PROCEDURE

The procedure in handling the pots was in minor details almost the same as in 1917. On the other hand, as has been mentioned, they were not under glass but under a canvas cover which was raised during the entire day except when showers occurred, permitting unmodified sunlight to reach the trees and also allowing much freer air circulation, the primary effect of which was, undoubtedly, to prevent the occurrence of excessive temperatures. While the extreme sun temperatures recorded were not materially lower than in 1917, the air temperatures were scarcely above those outside the shelter and were very much lower than in 1917, when they averaged 6° F. higher than the outside air.

The revolving table on which the pots were exposed was nearly 5 feet in diameter and made a revolution every two hours.

RESULTS

Amount of Transpiration Compared with 1917

The first point to be noted in Table VII is that the amount of transpiration in 1920 was very much less than in 1917.

On the basis of mean green weight it was 42.4 gm. in 1920, as compared with 123.0 in 1917. On the basis of leaf exposure it was 8.81 gm. in 1920, as compared with 20.6 in 1917. For leaf area we have no data in 1920. It is evident, however, that, considering the amount of water used in relation to size of trees, the transpiration was only one-third to two-fifths as great in 1920 as in 1917. This may be accounted for—

1. By a season of only about 147 days for the average tree in 1920, as compared with 203 days in 1917.

2. By reason of much lower air temperatures in 1920.

3. By reason of considerably less sunshine in 1920, but especially the lack in June, when the driest atmosphere usually prevails. The following data for whole months give the sunshine in recorded minutes:

	1917.	1920.
May	8,622	9,598
June	15,807	8,903
July	12,932	11,310
August	10,496	11,704
September	10,442	11,695
Total	58,299	53,210

It is also probable that on account of the arrangement of the room the trees received a smaller percentage of the total sunshine in 1920.

4. The water of the soil was less readily obtainable in 1920.

TABLE VII.—*Actual water losses and transpiration in relation to size and growth in 1920*

Species.	Yellow pine.				Douglas fir.		
Pot No.	22	1	18	15	17	10	7
Transpiration:							
May 11 to 31	51.5	52.8	35.6	66.2	83.0	108.9	112.0
June	109.0	112.0	102.7	144.6	111.4	122.9	134.0
July	132.4	163.1	236.4	280.0	117.6	162.6	149.0
August	161.2	236.8	338.2	492.7	130.3	180.0	177.0
September	151.4	292.8	313.0	509.4	130.9	190.3	181.8
October (to day indicated)	(7) 31.6	(8) 80.5	(8) 73.7	(7) 105.9	(8) 47.8	(5) 32.1	(6) 39.7
Total for season	637.1	939.0	1,099.6	1,598.8	621.0	796.8	793.5
Correction, direct evaporation	79.4	79.6	79.6	79.4	79.6	77.7	79.0
Net transpiration	557.7	859.4	1,020.0	1,519.4	541.4	719.1	714.5
Transpiration:							
Grams per gram weight accretion—							
Green	715	231	185	151	501	.....	345
Dry	564	592	615	514	302	506	992
Grams per gram mean green weight	26.1	41.8	56.8	67.3	19.1	21.6	32.1
Grams per square centimeter leaf exposure	6.5	10.1	9.5	14.2	3.8	5.1	5.0

TABLE VII.—*Actual water losses and transpiration in relation to size and growth in 1920*—Continued

Species.	Lodgepole pine.			Engelmann spruce.			
Pot No.	3	23	24	9	19	12	11
Transpiration:							
May 11 to 31	64.5	83.7	94.5	125.9	93.5	99.6	181.8
June	154.8	246.6	243.2	177.3	143.3	127.9	327.7
July	196.3	548.3	607.1	212.1	149.4	194.1	607.8
August	394.0	736.0	923.6	341.3	240.5	272.5	894.5
September	435.1	627.5	708.9	363.6	224.8	227.8	926.0
October (to day indicated)	(8) 103.1	(6) 106.8	(6) 118.7	(5) 56.2	(7) 42.3	(5) 29.8	(5) 123.8
Total for season	1,347.8	2,338.9	2,696.0	1,276.4	893.8	951.7	306.6
Correction, direct evaporation	79.6	79.0	79.0	77.7	79.4	77.7	77.7
Net transpiration	1,268.2	2,259.9	2,617.0	1,198.7	814.4	874.0	2,983.9
Transpiration:							
Grams per gram weight accretion—							
Green	148	204	195	138	162	149	140
Dry	701	802	688	450	431	420	494
Grams per gram mean green weight	52.9	82.0	66.9	74.6	103.8	86.1	45.3
Grams per square centimeter leaf exposure	11.4	19.0	15.4	11.6	12.0	10.4	10.1

Species.	Limber pine.		Bristlecone pine.		Scotch pine.		Siberian larch.		All.
Pot No.	16	21	8	4	13	2	6	20	.....
Transpiration:									
May 11 to 31	131.2	79.2	57.3	74.3	81.7	39.1	71.9	69.2	.....
June	151.2	77.2	105.3	128.1	149.1	121.6	158.1	152.8	.....
July	321.3	84.1	128.8	114.5	183.4	231.0	236.2	165.6	.....
August	484.4	112.9	132.2	107.5	194.7	416.0	457.0	322.4	.....
September	.....	.....	.....	.....	.....	.....	(27)	(27)	.....
October (to day indicated)	534.4 (8) 132.5	125.2 (7) 31.4	108.4 (3) 7.1	87.6 (3) 6.7	212.7 (6) 38.2	454.7 (8) 102.1	123.5	152.8	.....
Total for season	1,755.0	510.0	539.1	518.7	859.8	1,364.5	1,046.7	862.8	.....
Correction direct evaporation	79.6	79.4	76.0	76.0	79.0	79.6	70.3	70.3	.....
Net transpiration	1,675.4	430.6	463.1	442.7	780.8	1,284.9	976.4	792.5	247,940
Transpiration:									
Grams per gram weight accretion—									
Green	170	.....	230	168	420	191	243	311	198
Dry	773	4,785	317	434	409	464	333	257	522
Grams per gram mean green weight	24.4	13.5	15.1	32.0	25.6	52.6	94.0	49.7	42.4
Grams per square centimeter leaf exposure	7.0	3.6	3.7	6.3	5.0	11.9	10.0	6.0	8.81

On the basis of the growth made, the transpiration is also less in 1920 than in 1917, though not so strikingly so. For green-weight accretion the figures are 198 and 263, and for dry-weight accretion 522 and 880, respectively, for 1920 and 1917. In other words, for green-weight accretion it required 75 per cent as much water in 1920 as in 1917, and for dry-weight accretion 59 per cent as much. The difference between these two percentages and between the two years may be due largely to the fact that very little foliage falling in 1917 was salvaged and accounted for, while in 1920 this was carefully done. However, it is believed the amount dropped by the trees in 1917 was relatively very small and insufficient materially to affect the results.

It seems fairly evident that the transpiration per unit of growth is a more stable quantity than that per unit of leaf exposure or whole mass, in spite of the fact that the former is very much dependent on the whole leaf area functioning.

Water Requirements

Comparing now the species, as was done for the data of 1917, we have them in 1920 aligned as in Table VIII.

TABLE VIII.—*Comparison of different species as to water requirement*

Species.	Transpiration per unit of dry-weight accretion.	Probable error in average.
	Gm.	*Gm.*
Limber pine	2,779	850
Lodgepole pine	730	18
Douglas fir	600	160
Yellow pine	571	5
Engelmann spruce	441	6
Scotch pine	436	23
Bristlecone pine	376	49
Siberian larch	295	32

Resistance to Transpiration

On the basis of leaf exposures we have a very different arrangement (Table IX).

TABLE IX.—*Comparison of species as to resistance to transpiration*

Species.	Transpiration per square centimeter leaf exposure.	Probable error in average.
	Gm.	*Gm.*
Lodgepole pine	15.27	0.49
Engellmann spruce	11.12	.21
Yellow pine	10.08	.66
Scotch pine	8.45	2.91
Siberian larch	8.00	1.69
Limber pine	5.30	1.44
Bristlecone pine	5.00	1.10
Douglas fir	4.63	.39

EXPLANATION OF RESULTS

On thorough consideration of the meaning of the results which have been given above for both 1917 and 1920 tests, we come to the conclusion that neither method of comparing the species is very satisfactory when the number of individuals involved is insufficient to cover all possible variations. In these tests considerable variation in growth rate is to be expected as the result of more or less incomplete recovery from transplanting. The small spruces, for example, in 1920 showed no delay in starting new growth; the single large spruce came on satisfactorily after considerable delay; one large limber pine grew vigorously while the smaller one did not extend its terminal or branch buds over one-fourth

inch and put on no new foliage. None of the Douglas firs grew vigorously in 1920 while all of them dropped a good deal of their old foliage.

The water requirements and the rate of transpiration per unit of mass or leaf exposure are closely interrelated, it will be seen, for the following obvious reasons:

1. New shoots undoubtedly transpire more freely than old foliage.

2. When a plant is not growing it seems to transpire relatively little, either because it can not obtain the water or, possibly, because it has closed its stomata.

3. It therefore follows that the amount of transpiration per unit of mass or leaf exposure may be very much affected by the amount of growth made.

4. And it is equally apparent that the transpiration per unit of growth may be somewhat dependent on the total amount of foliage functioning, though it must be conceded that so long as the old foliage transpires, it probably is also capable of some photosynthesis, and therefore contributes to growth.

The important point is to recognize that an extreme case of poor growth may throw the specimen very high in one list and very low in the other list (for example, Pot 21 in 1920). It seems, therefore, only reasonable to eliminate from both records the individuals which have apparently not performed normally in the matter of growth. As the basis for normalcy is so meager, we can not bring ourselves to the elimination of any trees except one limber pine in 1917 and another in 1920.

On the other hand, what is true of individual trees affects the relations of the species. Apparently, small spruces are capable of a generally larger accretion percentage than similar trees of our other native species. As has been pointed out, this would be a very important factor in competition. Its bearing on absolute water requirements and drought resistance is not so plain, and we have had serious misgivings as to the desirability of comparing the species, in their moisture relations, on this basis. Nevertheless, it is fairly apparent that a high growth percentage in itself denotes something of superiority in the relation of the tree to its environment. It indicates either that the tree has some peculiar ability to make use of the available light or that it is more capable than others of supplying the water, or the carbon dioxid, in just the right amount to make photosynthesis effective. If either of the latter is a factor in the result, we may say either that the plant has superior ability to obtain water or that it has superior ability to retain it while keeping the stomata open for the ingress of carbon dioxid. There is left, therefore, little doubt in our minds that the tree of low "water requirement" as related to growth is in fact the tree which has the superior control over its water supply.

It is, therefore, important to compare the species on the basis of the growth made, in order to understand the marked differences between the absolute transpiration rates in 1917 and 1920, which leave the relations of the species so confused.

On comparing further Tables III and VIII, it is seen that with one or two exceptions, namely, yellow pine and bristlecone pine, the water requirements as determined in 1917 and 1920 are not so divergent that we need hesitate to combine them to obtain more effective averages, and it seems best to use the value for each tree in obtaining the mean. There are also given the mean growth percentages for each species. In figure 2 the general relation between growth rates and water requirements is plainly shown—a relation that seems logically unavoidable.

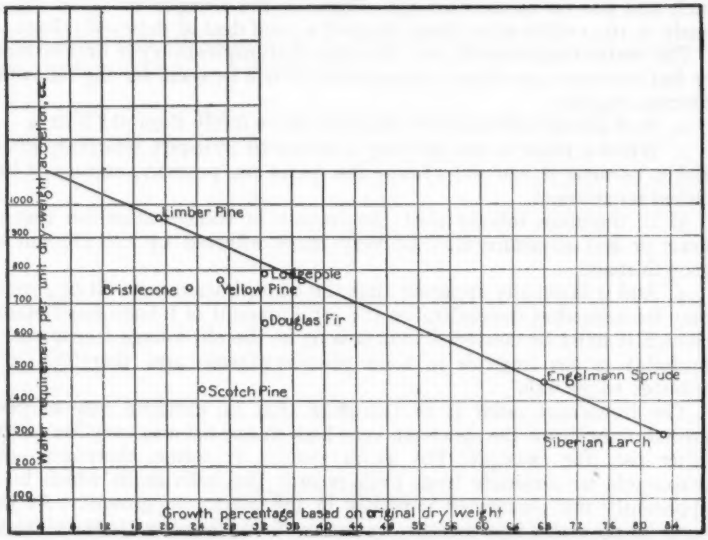

FIG. 2.—General relationship between water requirements and growth rates for all species in 1917 and 1920.

TABLE X.—*Water requirements and growth in 1917 and 1920*

Species.	1917			1920			Average water requirement.	Average growth percentage.[a]
	Number of trees.	Water requirement.	Growth percentage.	Number of trees.	Water requirement.	Growth percentage.		
		Gm.			*Gm.*		*Gm.*	
Limber pine	1	1,144	31.0	1	773	7.5	958	19.2
Lodgepole pine	2	954	46.6	6	730	27.8	786	32.5
Yellow pine	2	1,555	32.4	8	571	25.6	768	27.0
Bristlecone pine	2	1,110	31.3	2	376	14.7	743	23.0
Douglas fir	2	684	64.9	3	600	11.0	634	32.6
Engelmann spruce	2	525	75.2	9	441	66.4	456	68.0
Scotch pine	.....	.....	.....	2	436	24.6	436	24.6
Siberian larch	.....	.....	.....	2	295	83.5	295	83.5

[a] Dry accretion related to dry weight at beginning of season.

At least two important points are gained by the combination of the 1920 with the 1917 data. The position of limber pine, as the least effective user of water, is more nearly established, and lodgepole pine is brought into this class, where our empiric estimates would place it, as we shall see later.

But the main reason for presenting the growth data in Table X is to explain the positions of the species as given in Table IX. It is seen that Engelmann spruce maintained in 1920 almost as high a growth rate as in 1917, and this explains its appearance as a relatively extravagant user of water on the leaf-exposure basis. Conversely, limber pine and Douglas fir both appear as conservative users of water in 1920, evidently because they were relatively inactive.

In combining, then, the data for the two years on transpiration per unit of leaf exposure, we shall not lose sight of the fact that the positions of Douglas fir and limber pine, at least, and to a lesser extent that of bristlecone pine, are determined by relative inactivity in 1920, and that they really belong higher in the scale than here shown. There are possible means of making allowance for this influence of growth activity on the total transpiration, but they are so purely arbitrary that we hesitate to use them.

TABLE XI.—*Mean resistance to transpiration, 1917 and 1920 (growth data repeated to explain relative positions)*

Species.	Mean transpiration per square centimeter leaf exposure.	Mean growth percentages.
	Gm.	
Lodgepole pine	17.35	32.5
Yellow pine	14.96	27.0
Limber pine	14.15	19.2
Bristlecone pine	13.10	23.0
Engelmann spruce	11.97	68.0
Douglas fir	9.50	32.6

That these relations of the species are not fixed and might easily be modified by additional data or consideration of different climatic varieties is fairly apparent from the divergence of the individual results. On the same basis as Table XI, the three lots of yellow pine studied in 1920, each of which maintained a healthy condition and performed, relatively, much as it had been performing in the nursery, gave the following results:

Variety.	Transpiration per square centimeter leaf exposure.	Growth percentage.
	Gm.	
Harney, S. Dak.	8.3	11.7
Bitterroot, Mont.	9.5	19.5
Tusayan, Ariz.	14.2	27.0

There is every reason to believe these results were normal and express something of varietal difference. It is seen that the high growth rate of the Arizona form was accomplished with the extravagant use of water. Whether this is generally true of the more southern forms our data are inadequate to determine. Rather similar differences occur with lodgepole pine, but here the Wyoming form is the most extravagant and rapid growing.

ABILITY OF THE SPECIES TO OBTAIN WATER

A very considerable amount of light is thrown on the meaning of Table XI and our other discussions by considering the water requirements of each species in 1920, with the considerably less abundant water supply, as a measure of the ability of each to extract water from the soil. Thus, in comparing Tables III and VIII it was particularly noted that bristlecone and yellow pines, which in 1917 were more generous users of water, in 1920 took very low positions in the scale. The relative changes are indicated in the following table, where the use in 1920 is expressed as a percentage of the use in 1917. Only the better growing limber pines are considered. Comparison is made on the basis of Tables III and VIII and Tables IV and IX.

Species.	Relative transpiration in 1920.	Species.	Relative transpiration in 1920.
	Per cent.		*Per cent.*
Engelmann spruce	77.2	Limber pine	48.5
Lodgepole pine	66.2	Yellow pine	33.0
Douglas fir	57.6	Bristlecone pine	28.8

In some degree these observations are corroborated by the data in Table XII in which is shown the change in relative transpiration rates through a period in which the last pot listed for each species was given additional water. For the periods represented by September 28, 29, and 30, the water in each pot was at 70 gm. below standard, and except for the one pot of each species, the same amount was maintained on succeeding days. On the morning of September 30 the amounts in the special pots were increased 50 gm., and on October 1 they were brought up to standard. After this, they were not again watered until dried out to the original basis. If, then, the availability of the water, which was thus increased from about 0.461 to 0.594, as described under the heading "soil," has a bearing on the amount transpired, its effect should in all cases be apparent in the transpiration recorded October 1 and 2. In the table is shown what was the prevailing relative amount for the special tree, compared with others of the same species for the three days when water contents were the same. The relative amount on October 2 is then shown as a percentage increase.

It is to be noted that after October 2 the relative rates of the specially watered trees steadily declined, and in some cases went below the previous rates, until the day indicated by "W," when the first fresh water would take effect. This is plainly due to exhaustion of water close to the roots and is a commentary on the importance of transport within the soil.

TABLE XII.—*Effect on transpiration of increasing the available water a*

TABLE XII.—*Effect on transpiration of increasing the available water* [a]

Species.	Pot No.	Transpiration measured on the morning of—									Average relation Sept. 28 to 30.	Percentage increase Oct. 2.
		Sept. 28.	Sept. 29.	Sept. 30.	Oct. 1.	Oct. 2.	Oct. 3.	Oct. 4.	Oct. 5.	Oct. 6.		
Yellow pine	1	11. 0	10. 8	10. 3	10. 5	12. 9	12. 0	13. 4	9. 5	11. 6		
	18	10. 6	9. 8	12. 0	10. 8	11. 1	10. 8	13. 1	9. 7	10. 1		
	22	5. 4	4. 5	5. 1	4. 9	5. 9	5. 5	5. 6	4. 5	5. oW		
	15	18. 7	17. 4	19. 5	21. 2	28. 1	20. 3	19. 4	10. 5	13. 6		
Relative amount in No. 15 [b]		. 693	. 694	. 712	. 810	. 940	. 718	. 605	. 443	. 510	0. 700	34. 4
Douglas fir	7	6. 3	6. 4	6. 3	7. 0	8. 0	8. 7	8. 6	6. 5	7. 9		
	10	6. 8	5. 7	7. 5	6. 2	8. 0	8. 6	8. 9	6. 6	.....		
	17	4. 3	3. 7	5. 1	6. 4	7. 0	8. 0	7. 4	5. 8	.....		
Relative amount in No. 17 [b]		. 328	. 306	. 370	. 485	. 437	. 462	. 423	. 443	.....	. 335	{ 44. 8 / 30. 5
Lodgepole pine	3	15. 6	14. 2	16. 4	13. 7	17. 2	14. 7	19. 3	12. 2	14. 0		
	23	21. 9	22. 9	23. 5	20. 1	24. 6	21. 1	24. 5	17. 7W	18. 9		
	24	23. 1	19. 9	23. 8	24. 4	32. 6	27. 3	23. 4	16. 5	18. 9		
Relative amount in No. 24 [b]		. 616	. 537	. 597	. 722	. 780	. 763	. 535	. 552	. 593	. 583	33. 9
Engelmann spruce	9	12. 1	12. 6	13. 8	12. 3	14. 7	13. 3	15. 3	12. 9	.....		
	12	7. 2	6. 9	7. 3	6. 4	8. 1	6. 5	8. 3	6. 9	.....		
	19	6. 9	6. 8	7. 6	7. 2	8. 1	6. 8	8. 7	6. 5	.....		
	11	32. 4	30. 5	35. 5	34. 5	40. 7	32. 9	27. 9	22. 3	.....		
Relative amount in No. 11 [b]		1. 237	1. 160	1. 237	1. 332	1. 318	1. 237	1. 158	1. 180	.....	1. 211	8. 8
Limber pine	21	4. 5	3. 6	4. 2	4. 5	6. o	5. 3	5. 5	4. oW	5. 5		
	16	16. 9	16. 4	17. 8	20. 2	28. 8	24. o	21. 3	13. 1	16. 3		
Relative amount in No. 16 [b]		3. 76	4. 56	4. 24	4. 49	4. 80	4. 53	3. 88	3. 28	2. 96	4. 19	14. 5

[a] Last pot for each species increased 50 gm. effective October 1 and 20 gm. more effective on morning of October 2.
[b] The amount in the pot receiving extra water is expressed as a ratio to the total in other pots of same species.

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From the data in Table XII we see that the limber pine was little stimulated, probably because its transpiration is always moderate. The spruce was still less affected, apparently because it is always able to satisfy its needs. Lodgepole, Douglas fir, and yellow pine were about equally helped and seemed greatly invigorated. The relatively high transpiration of the fir on October 1 and 3 can be accounted for only by an error in weighing.

To a certain extent, these performances may be accounted for by the root habits of the trees. It is possible that the finely divided and numerous roots of the spruce give it immediate control over so much more soil that it exhausts the available water much less quickly than those species which usually develop only a few coarse roots. To some extent this would also explain the ability of limber pine to obtain its water more steadily. On the other hand, either lodgepole or Douglas fir ordinarily has much better roots than yellow pine, yet these three were about equally stimulated by a heavy addition to the water supply.

On the whole, this matter is only suggestive and does not, we believe, explain the relative behavior of the species. That Engelmann spruce possesses a remarkably great ability to supply itself with all the water that is needed under the most trying circumstances, and that this ability is exceeded, among the species studied, only, possibly, by that of Siberian larch, seems proved beyond a shadow of doubt. This is plainly shown in the day-to-day records where, if there is a marked contrast in the amount of sunlight on two succeeding days, or in other conditions conducive to high transpiration, it is the spruce which is invariably able to live up to these conditions most fully. Thus we are enabled to say quite confidently that the relatively high rate of transpiration of spruce on cloudy days, as shown by Table V, truly expresses an ability to make use of all available light and does not signify that this species is unable to meet the conditions which cause high transpiration from all the species.

SUMMARY

It has seemed desirable to go into this matter fully on account of the complicating features introduced by the radically different results secured in 1917 and 1920, and in order that we might not deceive ourselves as to the true meaning of the results. It has been necessary for us to go through with this analysis in order to reach a conclusion, and it is hopeless to expect the reader to reach a conclusion by any other process.

It now becomes fairly apparent that transpiration is very much dependent on water supply and that the relatively low water use of some of the species in 1920, when the water supply was maintained at a low level, is not to be considered as a virtue but rather as evidence of a lack of ability to supply needs. And, even though in some cases growth may not have been seriously impaired by the inability of certain species to keep the leaves well stocked with water, yet it is perfectly evident that the species which show this inability in the most marked degree would soonest succumb in time of real drought or in the usual autumnal drought that occurs where there is strong competition.

There are, apparently, two slightly different problems to be considered in comparing the species. The one has to do with the relative requirements of different tree species of a unit size. The other has to do with

the amount of water required during the production of a unit of growth. Both relations are important in ecology. But, except with the weed trees, limber and bristlecone pines, we have found no essential difference in comparing the species on the two bases. To what extent a low water requirement means great drought resistance we shall see later. Apparently there is not a great deal of difference between the species at the minimum water point. There is evidently a great difference in their activity or vigor under better conditions, and this, perhaps, is the most important point we have brought out.

The important consideration is that the additional data secured in 1920 have not materially altered our conception of the physiological qualities of the species, which are best indicated relatively by Table III. It is true that in 1920 lodgepole pine used relatively more water and made less vigorous growth, so that now, by either Table X or XI, it appears as a more extravagant demander than yellow pine. Likewise Engelmann spruce kept up its rate in 1920 more nearly than Douglas fir and hence appears more extravagant. These facts, however, merely confirm the belief that the species which under favorable moisture conditions is most conservative is best able under all conditions to satisfy its needs. The reason for this will be more apparent after considering sap density and its osmotic bearings.

To summarize, briefly, for the species, what it is believed is shown by the preceding data and discussions:

1. Limber pine: Very slow growing, but also very conservative in the use of water. Represents highest development in structural protection against atmospheric conditions, but probably poor development in relation to the soil. Not adapted for competition.

2. Bristlecone pine: Not quite so far developed as limber pine in any respect mentioned.

3. Yellow pine: Relatively slow grower and has little protection against losses; consequently from either standpoint its water use is very high. Shows little ability to cope with drought conditions. Arizona form more vigorous and equally extravagant of water.

4. Lodgepole pine: More rapid grower than yellow pine.

5. Douglas fir: Apparently adapted to conserve water but growth rate not nearly equal to spruce, possibly being in these tests more adversely affected by transplanting because of the relatively long roots, which are characteristic, and their small numbers.

6. Engelmann spruce: Most highly developed of our native species to make use of all conditions of environment in vigorous growth. Is conservative of water and low in water requirements for growth. These characteristics may partly explain its shade tolerance and its success in competition.

7. Siberian larch: Although little studied, seems to be developed even beyond spruce in all particulars.

8. Scotch pine: Stands about midway between our pines in transpiration rate and lower than any of them when growth is considered. Seems to be developed along lines of spruce and fir for alpine conditions. It should be remembered we are speaking only of the Riga form.

SAP DENSITY AND THE VARIATION IN TRANSPIRATION RATES

Sometime before the transpiration tests which have just been described were made in 1917, carefully conducted drying tests on green and partly

dried lodgepole cones, in a calorimetric kiln, had clearly demonstrated that the amount of heat required to extract a gram of water from cones was not 536 calories but an amount always in excess of that, which increased rapidly as the amount of sap in the cones decreased through preliminary drying. This apparent increase in the latent heat of vaporization, it was thought after a study of the physical chemistry of solutions, might be related to the phenomena of rising boiling points and decreased vapor pressure with increases in the concentration. Unfortunately, no direct experimental work on this problem had been done, so far as I have been able to learn to date, and a number of physicists consulted agreed that in their interpretation of the theory of solutions a solute could not increase the latent heat of vaporization of the solvent.

The writer, in the most dependable tests it has been possible to make, has found that at the respective boiling points of various concentrations of common salt in water, the latent heat of vaporization decreased slightly with increased concentration, up to the point of saturation. Making allowance for the higher boiling point of the concentrated solution, it would appear that for a given temperature the latent heat of vaporization was practically a constant. In these tests an electric immersion heater was employed for the heat supply, the wattage being precisely measured; the evaporation was directly measured by weighing the solution; and allowance was made for direct radiation from the solution and vessel.

The greatest objection to these tests, or to any that we have so far been able to devise, lies in the difficulty of maintaining a constant temperature with a constant and measurable heat supply, at a relatively low temperature such as plant tissues may experience, and also at a relatively low temperature such that the radiation factor is not a great possible source of error. Until these difficulties are overcome we can hardly say that the problem of the latent heat of vaporization as it relates to plant evaporation has been satisfactorily treated.

On the other hand, it is a fairly simple matter, at either high or low temperatures, to determine that the rate of evaporation is very materially reduced with increase in concentration when the source of heat is outside the solution. This would make it appear that there may be a problem in conductivity quite as important as, if not more important than, that relating to latent heats. The resistance to drying, shown by rather concentrated solutions such as sirup, is quite well known. As an illustration of what we mean by heating from the outside, let us take the case of two identical evaporating dishes placed over a steam bath. The steam is constantly in contact with the bottoms of the dishes. It may not, however, give up its heat unless the surface of the dish is being cooled by radiation above or evaporation of the liquid in the dish. Such an exposure can not possibly give any indication of the quantity of heat utilized in evaporating from the dish.

Under such circumstances as these the writer found the evaporation from a saturated salt solution to be less than one-twentieth as rapid as that from pure water.

Similarly, exposing a number of test tubes mainly to the heat of the air in contact with them, the rates of evaporation were found to be depressed by somewhat dilute saline and sugar solutions.

Vessels and bottles in which the contents have been heated primarily by the rays of the sun have not shown any consistent depression of the evaporation rates due to solutes.

The available facts, then, which have a bearing on the possible influence of sap concentration on the rates of evaporation from similar bodies are:

1. At any given temperature the vapor pressure over a solution decreases as the concentration increases, indicating that the solution does possess a stronger hold on the molecules than does the pure solvent and that therefore the solute may at least decrease the rate of evaporation. There is nothing in the quantities involved, however, to indicate that this might be an important factor within the limits of cell-sap concentrations.

2. Calorimetric tests on the heat required for drying cones indicate an increase in the latent heat of vaporization as the concentration of solutions in the cone cells increases. Even admitting that the large number of tests puts the facts practically beyond question, there may be here a case of adsorbed water rather than a case of solutions, and with the molecular affinities and possible latent heats of the former we are not, just at present, concerned.

3. Carefully conducted tests on free saline solutions indicate that the latent heat of vaporization is not appreciably affected by concentration or at least not more so than might be deduced from paragraph 1.

4. Observations on the heating and evaporation of solutions by low-temperature, exterior sources indicate an inability, increasing with concentration, to absorb and transmit such heat in a manner conducive to evaporation. We shall not attempt to go into the theory of this. The important fact is that the heat of the air, and sunlight so far as it is absorbed by the exterior walls of the leaf or the interior cell walls, may be relatively ineffective in producing evaporation from a concentrated as compared with a dilute solution, while, apparently, such rays as were directly absorbed within the solution would be about equally effective in all cases. It goes almost without saying that, if such absorbed heat does not produce evaporation, it must increase the temperature of the leaf until a point is reached where absorption and radiation balance.

In view of these facts, when, at the close of the transpiration tests, it was discovered that the several species showed such unaccountable and surprising differences in transpiration rate, with respect to growth or mass or leaf area, the first thought was that they must exhibit differences which could be expressed in the qualities of the solutions from which the evaporation of water takes place.[5] This thought was too hastily transformed into action by igniting the specimens which had served for the transpiration tests, in the expectation that the ash weights would be an index to the solutes in the plants and the densities of their cell solutions. This supposition was, of course, erroneous in taking no account of the soluble carbohydrates as well as some of the mineral oxids which would be lost in ignition and which comprise the greater mass of the solutes. As indicated in Table I, the ash percentages are irregularly variable and are found to bear no relation to transpiration rates.

Having destroyed the best source of information on the physical qualities of the original specimens, the next step was to obtain specimens as nearly as possible like those used in the transpiration tests. This was done by securing trees of the same classes as those taken from the nursery in the spring of 1917 which had spent the growing season in the nursery.

[5] The writer wishes to acknowledge the very helpful suggestions of the article by Barrington Moore (*13*), which was received in galley proof at such a time as to aid very materially in solving the current problem, and which reviews a number of the more recent researches on this problem.

The specimens were collected on December 3, 1917, before the ground was frozen, and when there had as yet been no drying winds. From three to eight trees of each species were taken so as to secure a considerable mass of material. The tops were cut off at the root collar and those parts only were used.

PROCEDURE IN DETERMINING SAP DENSITY

The necessary data for determining the momentary density of the sap in a plant appeared to be—

(1) The weight of the green material, determined as quickly as possible after the material was collected.

(2) The weight of the soluble matter leached out with an abundance of water and evaporated.

(3) The weight of the insoluble pulp, oven-dried.

By adding together (2) and (3) and deducting from (1), the original amount of water (and other solvents) in the plant is obtained, and this, when divided into (2), gives the sap density, usually expressed as a percentage.

In these original tests the plant material for each species was ground to a pulp, and these pulps were allowed to stand in cold water of about 10 times the pulp volume, 3 waters being used for each. Finally the pulp was all accumulated on a filter and dried with the filter paper. The aqueous solutions were evaporated at temperatures not exceeding that of boiling water. In all cases the so-called "sugars" thus secured, after becoming dry, were not wholly resoluble, indicating that colloidal matter had been included and had passed through the filters. This matter was a small proportion of the total solids, however, and may be assumed to have equally affected all samples.

RESULTS

The sap density percentages obtained for the nursery seedlings, in the first tests made, were as shown in Table XIII, in which the water requirements are again given. Figure 3 shows that the sap densities and water requirements plot a curve which is remarkably perfect, considering the changes that have been noted in relative transpiration rates during the season, and the somewhat questionable value of the water requirement for limber pine, which must be based on the performance of only one specimen.

TABLE XIII.—*Sap densities and water requirements in 1917*

Species.	Sap density in material of Dec. 3, 1917 (tops only).	Water requirements of trees in transpiration tests.
	Per cent.	*Gm.*
Limber pine	19.6	1,144
Yellow pine	21.8	1,555
Bristlecone pine	22.4	1,110
Lodgepole pine	23.2	954
Douglas fir	27.9	684
Engelmann spruce	29.5	525

At first thought it does not appear probable that the density of the spruce sap, which is only one-half greater than that of yellow pine, is sufficient to reduce the relative water loss of spruce to one-third or one-fourth of that of the pine. It is not necessary to assume that the small water loss of spruce, as related to its photosynthetic activity and amount of growth, is entirely the direct result of the physical properties of the dense sap of this species. To determine how the result may be brought about, all the following factors must be given consideration:

1. Higher sap density means less evaporation per unit of available heat.

2. Higher sap density means higher leaf temperatures before evaporation can take place at a given rate, with the possibility that in sunlight the leaf may become warmer than the air and therefore lose heat by radiation and conduction.

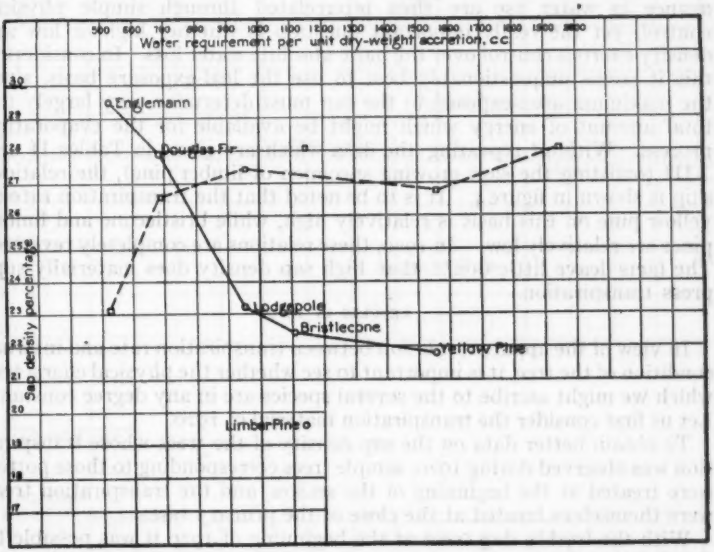

FIG. 3.—Water requirements in 1917 transpiration tests compared with sap densities on nursery specimens, December 3, 1917 (solid line) and sap densities in natural habitats, December 30, 1917 (broken line).

3. Higher sap density, by permitting higher leaf temperatures, should facilitate the photosynthetic process, thus relatively reducing the water requirement.

4. It must be equally true that greater photosynthetic activity or capacity will tend toward higher sap density as well as increased growth.

When, therefore, the question is asked, "Is the high sap density of spruce a direct cause of its low transpiration rate, or is the former merely a concomitant of greater photosynthetic activity, and is this last the really important physiological characteristic?" we are compelled to reply that the three things are so interdependent that all are equally causes and effects. It is left almost beyond question, however, that of the species we have considered the spruce represents the highest development and that this development is expressed in the highest growth rate (first columns of Table X) in the greatest current accumulation of soluble carbohydrates at the time of the December examination and in the most

effective use of water throughout the season. On the other hand, when we consider the low sap density of limber pine and its low water requirement in relation to either mass or leaf area, we obtain a suggestion that transpiration may be controlled by mechanical means rather than through the physical properties of the sap and that such control indicates a low state of development because it inevitably means the sacrifice of the absolute growth rate. Yellow and lodgepole pines, with relatively low sap densities, appear not to exert the mechanical control over transpiration and are, as a result, perhaps more fastidious as to growing conditions than limber and bristlecone pines.

While it seems important to have demonstrated that among the species of approximately equal development from the forester's standpoint, growth, photosynthetic activity, sap density, and the relative extravagance in water use are thus interrelated through simple physical control, yet the really important question is whether high or low sap density exerts a control over the more absolute water loss. In considering this it seems unquestionably best to use the leaf-exposure basis, since the maximum area exposed to the sun must determine very largely the total amount of energy which might be available for the evaporating process. Without repeating the data which are given in Tables II and XIII (omitting the slow-growing specimen of limber pine), the relationship is shown in figure 4. It is to be noted that the transpiration rate of yellow pine on this basis is relatively high, while bristlecone and limber pines are relatively low. In 1920, these relations are completely reversed. The facts leave little doubt that high sap density does materially suppress transpiration.

RESULTS IN 1920

In view of the apparent relation between transpiration rate and internal condition of the tree, it is important to see whether the physical characters which we might ascribe to the several species are in any degree constant. Let us first consider the transpiration material of 1920.

To obtain better data on the sap density of the trees whose transpiration was observed during 1920, sample trees corresponding to those potted were treated at the beginning of the season, and the transpiration trees were themselves treated at the close of the primary test.

With the freshly dug trees at the beginning of 1920 it was possible to grind and treat the whole plants in very much the same way as the tops were treated in December, 1917. Owing to the large number of lots involved, however, four sets of sample trees were merely dried, and it is necessary to deduce their approximate sap densities from other results for the same species.

In the fall examination it was considered of first importance to determine the dry weight of the trees, as a measure of growth, without the risk of losing any material or whole results through accidents. The trees were, therefore, first oven-dried. It was several months before opportunity presented itself to bring out this dried material, grind it in a mortar, make the extractions of sugars, and again dry the leached pulp. In this case no attempt was made to evaporate and weigh the sugars. This method is probably open to the criticism that a longer period is required to secure the same degree of leaching of solutes that may be expected with green material, and also that the drying of the material probably coagulates and holds some collodial matter that would, from green material, pass off with the solutes. This may account in part for the relatively low sap densities found at the end of 1920.

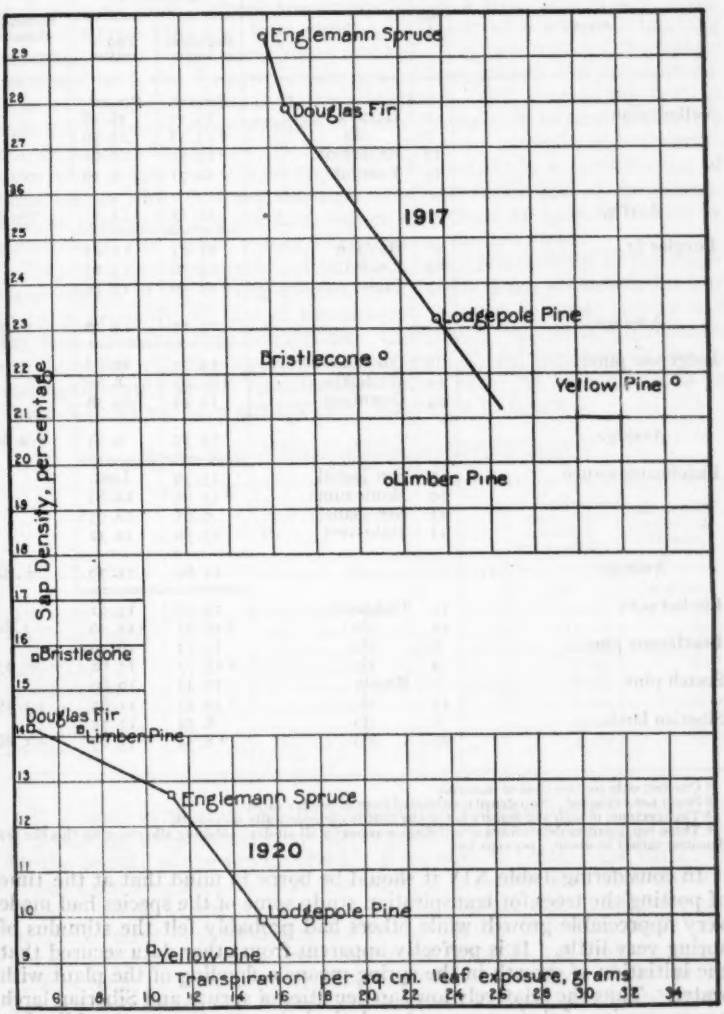

FIG. 4.—Relation between sap densities and transpiration rates on basis of leaf exposures.

The results of the two determinations are shown in Table XIV.

TABLE XIV.—*Sap density of trees in 1920 transpiration tests (whole plants)*

Species.	Pot No.	Source.	Sap density at—		Seasonal change.
			Beginning of season.	End of season.	
			Per cent.	*Per cent.*	
Yellow pine	22	Harney	13. 15	16. 18	
	1	.....do	[a] 13. 15	15. 59	
	18	Bitterroot	[b] 13. 50	13. 01	
	15	Tusayan	12. 71	9. 30	
Average			13. 13	13. 52	+0. 39
Douglas fir	17	Hayden	15. 24	15. 63	
	10	Leadville	14. 78	13. 25	
	7	Pike	[b] 15. 50	13. 71	
Average			15. 17	14. 20	−. 97
Lodgepole pine	3	Colorado	14. 72	10. 60	
	23	Washakie	[b] 14. 65	8. 80	
	24	Gunnison	14. 49	10. 38	
Average			14. 55	9. 93	−4. 62
Engelmann spruce	9	San Isabel	13. 70	Lost.	
	19	Montezuma	[b] 13. 65	12. 84	
	12	San Isabel	9. 91	12. 93	
	11	Unknown	13. 50	12. 32	
Average			12. 69	12. 70	+. 01
Limber pine	16	Unknown	16. 05	13. 02	
	21	.....do	[a] 16. 05	[c] 15. 36	−1. 86
Bristlecone pine	8	.....do	15. 75	16. 29	
	4	.....do	[a] 15. 75	15. 68	+. 23
Scotch pine	2	Russia	10. 43	10. 80	
	13	.....do	[a] 10. 43	12. 65	+1. 29
Siberian larch	6	.....do	8. 54	13. 75	
	20	.....do	[a] 8. 54	[d] 15. 05	+5. 86

[a] One test only for this class of material.
[b] Sugar not extracted. Sap density estimated from green-dry ratio.
[c] The specimen of high sap density is the one that made practically no growth.
[d] These sap densities determined after foliage was nearly all air-dry. Making allowance for this the sap densities should be about 2 per cent less.

In considering Table XIV it should be borne in mind that at the time of potting the trees for transpiration study some of the species had made very appreciable growth while others had probably felt the stimulus of spring very little. It is perfectly apparent from other data secured that the initiation of growth in the spring means a flooding of the plant with water. Thus the relatively low sap densities of spruce and Siberian larch are expressive of their response to relatively low temperatures, while that of yellow pine is more indicative of a low condition throughout the winter.

In view of the fact that the stage of the seasonal advance had affected the several species differently, it is questionable whether the spring data, or the changes throughout the season, have any great value in our present comparisons.

On the other hand, if the sap densities in the fall may be taken as indicative of conditions existing during most of the season, certain striking facts are in evidence. It has been mentioned that the abundant moisture supplied the trees in the spring, and the relatively dry condition later on, may have induced the production of a great deal of new tissue which the trees were not, later on, able to supply with adequate building material. This would seem most markedly the case with all the lodgepoles and with the Tusayan yellow pine whose growth was so vigorous. It is believed, therefore, that the evidence is fairly convincing that in 1920 either the moisture was not sufficiently available to permit effective photosynthesis in some of the species or that the sunlight and temperatures were below par in effectiveness. Possibly it is a combination of these things which left sap densities very low at the end of the season. Still, in comparing the absolute values with those of 1917, it should be remembered the earlier values refer only to the tree tops.

These sap densities are found to bear a broad relationship to the water requirements of the several species, though this is not so well defined as in 1917, probably because of the seasonal changes in water supply and the less favorable light conditions. Comparing the mean post-season sap densities with the transpiration per unit of leaf exposure, however, and again omitting the questionable limber pine, we have the data in Table XV, which have already been illustrated in figure 4.

TABLE XV.—*Transpiration and sap densities in 1920*

Species.	Transpiration per square centimeter leaf exposure.	Mean post-season sap density.
	Gm.	*Per cent.*
Lodgepole pine	15. 27	9. 93
Engelmann spruce	11. 12	12. 70
Yellow pine	10. 08	9. 30
Scotch pine	8. 45	11. 72
Siberian larch	8. 00	14. 40
Limber pine	7. 00	14. 19
Bristlecone pine	5. 00	15. 78
Douglas fir	4. 63	14. 20

It is again evident in the 1920 results that the effectiveness of sunlight in producing evaporation from leaves must be very considerably affected by the density of the sap involved. If we were to balance the variations in one season against those in the other, it is readily seen that the relationship would be almost perfect. To what extent these variations may be due to error in determining either sap density or leaf exposure must remain a question until a great deal more material has been examined. It does seem certain, however, that the relative positions of the species, in regard to either sap density or transpiration rate, are by no means constant. The best that can be done at present is to accept average values for each species, as has been already done in considering the transpiration independently. The explanations, already made, of variations in growth in the two seasons should be considered in connection with the variations in sap density.

STABILITY OF SAP DENSITIES

It has already been indicated that the sap density of a given species is by no means constant. In order that progress may be made in the use of the sap density measure, or the osmotic pressure determination, as an indicator of the relations between plant and environment, it is extremely important to realize (1) that the tree may pass annually through a definite cycle of changes and (2) that the current sap density may be quite largely influenced by current atmospheric conditions as well as water supply. If the tree were always able to supply as much water as was demanded by the losses at its leaves, then only a cyclic change would be apparent, dependent on cell division and photosynthesis, or primarily on temperature and light conditions. It might be said, therefore, that the species which shows the least fluctuation with current atmospheric conditions is the one best able to supply any demand for water, and it would seem that this species would best endure a long drought in the soil.

As to the cyclic change in sap density, it would seem that the following describe the general conditions of the seasons which bring it about:

1. In the spring we have rising temperatures and increasing duration and intensity of sunlight, which alone would increase the osmotic pressure in favor of the plant as against the soil. Coupled with this there is usually an abundant supply of moisture in the soil immediately after its thawing, often augmented by the melting of large masses of snow and by heavy rains. These conditions cause a heavy inrush of water and, because the atmospheric demands are at this season very moderate, an extreme turgescence of the tissues already formed becomes not only possible but unavoidable. It is believed this turgescence is the foundation for cell expansion and division, from which the new tissues arise.

2. With the advance of spring and advent of summer the moisture supply of the soil is usually much reduced, but even if this were not the case the formation of new tissues might be checked because of the very great increase in transpiration, due both to greater atmospheric demands and water losses from the new succulent tissues already formed. In the conifers, outward evidences of growth usually disappear abruptly early in the summer. The period of warmest weather, then, is not one for the formation of new tissues, but for the creation of the materials with which those already formed may be built up and solidified. Fruiting, of course, also demands some of these materials. The sap density should, therefore, increase from the moment that cell division becomes negligible.

3. The autumn season in temperate climates is almost universally the least favorable as regards current precipitation, and at this season the soil water is most likely to have been completely exhausted. For this reason the amount that can be supplied is often inadequate for all the transpiring members, and we witness the drying and falling of all deciduous leaves and of the oldest ones on the evergreens. Unless the water supply in the leaves becomes so low as to prevent the chemical processes, photosynthesis in the evergreens is not checked, and sap densities may be expected to reach their highest points, partly because the actual amount of water present is less than at any other season.

4. While under some circumstances the winter season may be one of almost complete dormancy, with photosynthesis stopped by low temper-

atures and little light and the movement of water stopped by freezing of the soil, such a condition does not describe the winters of the region in which we are particularly interested in this study. Here the winter days are often warm and bright enough to permit possibly some photosynthesis in the evergreens and certainly very considerable transpiration. Such days may be followed by severe cold of sufficient duration to freeze the tree and the soil to a depth of 1 or 2 feet. These cold waves are not uncommonly followed by warm winds which immediately thaw the foliage and may make great demands on its moisture before the tree stems and the soil thaw out enough to provide a new supply. Thus, in extreme cases great injury may be done, and in the usual weather cycles the tree is subjected to very marked changes in water supply and in the sap density of its foliage. At this season the sap density, it will be readily seen, may depend very greatly on the exposure of the tree, since the primary factor in drying is wind. A tree growing in a dense stand with a northern exposure passes through much more moderate changes than one in an open stand where both sunshine and wind may have full effect.

Sap Density in Period of Vernation

We have already pointed out, in connection with Table XIV, the possible bearing of different responses to temperature on the comparative sap densities in the spring of 1920. In Table XV are given additional data which, with the explanatory notes, make a very clear case. It is not surprising to find that a control test made with nursery material collected June 1, 1918, when growth of most species had begun, shows complete disarrangement of the December, 1917, values.

The material was collected as before, complete aerial portions being taken. These were placed immediately in stoppered bottles and were exposed each to the others' vapor until June 18, in order that from the losses or absorptions some idea as to relative osmotic pressures might be gained. The exposure was not long enough to give more than an indication. After this period the material was dried in the bottles, then leached and redried. Spruce specimens were inadvertently omitted from this collection.

TABLE XVI.—*Sap densities of nursery specimens at beginning of growing season, 1918 (tops only)*

Species.	Age.	Number of tests.	Average sap density.	Relative osmotic pressure indicated by vapor transfers.
	Years.		*Per cent.*	*Per cent.*
Limber pine	7	2	15.54±1.37	0.978
Yellow pine	2–6	2	15.10±0.55	.998
Bristlecone pine [a]	7	1	20.37	.980
Lodgepole pine	4	2	12.22±0.53	.979
Douglas fir	6	2	16.91±0.41	.969
Siberian larch [b]	4	2	10.61±0.98	.972
Western white pine [a]	3	1	20.33	1.014
Scotch pine	3	1	13.48	.984
Aspen (new leaves)	0	1	14.18	1.012

[a] Buds not yet opened.
[b] One specimen with and the other without roots. The former showed the lower density.

The data indicate that the beginning of new growth had permitted the essential equalizing of sap densities, except with bristlecone pine and western white pine. It is difficult to see why the density for bristlecone pine should have remained high without giving it, apparently, a high osmotic pressure. The very thin sap of Siberian larch is accounted for by the advancement of its growth, which always begins earlier than that of any of the native species.

TABLE XVII.—*Sap density of trees in natural habitats after drying weather, December, 30, 31, 1918*

Species.	Site where collected.	Sap density.
		Per cent.
Limber pine	2 southwest slope	28.67
	6 northwest slope	29.86
	6 northwest slope	26.42
	12 ridge	27.08
	9 north slope	28.39
	All	28.08±0.45
Yellow pine	2 southwest slope	[a]25.64
	2 southwest slope	[a]29.52
	6 northwest slope	27.46
	4 east slope	25.30
	12 ridge	25.84
	All	26.75±0.59
Lodgepole pine	1 south slope	27.24
	8 ridge	27.61
	All	27.42±0.16
Douglas fir	2 southwest slope	27.35
	4 east slope	26.40
	12 ridge	28.10
	9 north slope	24.44
	All	26.57±0.56
Engelmann spruce	3 northeast slope	24.38
	5 bottom	22.78
	5 bottom	22.16
	All	23.11±0.51

[a] On the morning of Feb. 23, 1918, corresponding specimens showed 21.05 per cent and 25.12 per cent, respectively, for these two trees. The specimen of lower sap density in each case was from a young, vigorous tree; that of higher density from a low limb of an old tree badly infested with mistletoe.

On the whole, while the determinations of osmotic pressure are not complete, it is indicated that they were essentially the same when the sap densities were nearly the same. We may, therefore, feel safe in assuming that for other conditions relative osmotic pressures will be about proportionate to sap densities, which might not be the case if the solutes of different species were materially different in composition and molecular weights. A boiling-point test made with accumulated solutes from all the species shows that an osmotic pressure of about 19 atmospheres may be expected when the sap density is 20 per cent. Freezing-point deter-

minations have also shown that with all of our conifers a sap density of 1 per cent is approximately equivalent to 1 atmosphere of osmotic pressure, this relation holding at least up to 20 per cent. Such tests have not, as yet, been sufficient to bring out any consistent differences in the saps of different species.

Winter Sap Densities in Natural Habitats

Attention may now be turned to determinations of the sap density of trees growing in their natural habitats, as made at the end of December, 1917. The foliage specimens were collected on the afternoons of December 30 and 31, both days being warm and the soil not yet frozen in any instance at a depth of a foot. The preceding week had been warm and dry, with a high evaporation rate for that season. Hence it may be expected that the results will show the influences of different exposures.

All material was from limbs at a height of about 4½ feet above the ground.

In this, as in all the following cases where only foliage is sampled, the outer half or two-thirds of the needles was clipped off with shears in sections about one-half inch long. This material was leached before drying and was otherwise treated as the ground pulps had been.

When these results are compared with those obtained from nursery stock on December 3 (fig. 3), it is seen that a very great but regular difference in the value exists. The average sap density of limber pine has increased 8 per cent, of yellow pine 5 per cent, of lodgepole 4 per cent; that of Douglas fir has decreased 1 per cent and that of spruce 6 per cent. These changes form almost a straight line when plotted with the original sap densities as abscissae.

This shows that sap densities in a given species are subject to great variations, but it does not mean that they have no significance. The differences between these field specimens and the nursery trees growing under uniform conditions reflect the fact that the pines had lately been subjected to the strongest drying influences, while the fir in part, and spruce wholly, had recently been protected from any severe drying. Also, owing to the protection afforded the latter species at all times, they had probably never had the benefit of full light and, therefore, may not have accumulated as large a supply of carbohydrates as the pines growing in the open.

To assume from this that spruce or fir is not subjected in the field to drying stresses equal to those experienced by the pines, or that the former would not tolerate great stresses as well as the latter, is altogether erroneous. These tests were made before the soil was frozen and before the winter exposure had had opportunity to bring about any degree of equilibrium between different sites. It is greatly to be regretted that this series of specimens could not have been duplicated late in the winter.

Winter Sap Densities Near Timber Line

On the other hand, specimens collected at high elevations, on January 1, 1918, only a day or two later, tell a very different story, for here the soil was already deeply frozen, and the exposure to evaporation had been very severe for the preceding six days.

These results and others which show changes with season, weather, and soil conditions are given in Table XVIII.

TABLE XVIII.—*Sap densities in exposed situations, at high elevations, 1918*

Species.	Location.	Sap densities.			
		Jan. 1.	Feb. 6.	May 7.	May 18.
		Per cent.	*Per cent.*	*Per cent.*	*Per cent.*
Engelmann spruce	Station F–16	[a]37.30	31.29	[b]33.60	22.16
Bristlecone pine	do	30.75	28.68	[c]19.71	20.84
Limber pine	Station F–13	31.00	24.00	.....	.....
Bristlecone pine	Cabin Creek	30.56	30.37	[d]21.70	21.50
Engelmann spruce	Gulch near F–13	.....	23.66	.....	19.02
Alpine fir	do	.....	21.45	.....	20.62
Limber pine	Cabin Creek	.....	.....	.....	21.07
Engelmann spruce	Cabin Creek, edge of water.	.....	.....	.....	24.51

[a] Similar specimen obtained Jan. 15, 1921, from a very exposed site showed 33.5 atmospheres of osmotic pressure by freezing-point method with 27.7 per cent sap density.
[b] Twigs from which needles were clipped showed at same time 19.95 per cent.
[c] Twigs from which needles were clipped showed at same time 15.84 per cent.
[d] Twigs from which needles were clipped showed at same time 15.19 per cent.

At Station F–16 there is at all times a contrast between the spruce and bristlecone pine specimens, until May 18, when thawing had become general. This is probably not altogether a specific difference but is due in part to the fact that the spruce was located in a hollow which collected snow and did not permit even temporary thawing of the soil until very late, while the bristlecone was on high ground only a few feet distant, from which the snow is usually swept away, and which might, therefore, thaw in a brief period.

It may be a very significant fact that although the soil temperatures at Station F–16 on February 6 were 21.5° F. at 1 foot, and 23.6° at 4 feet (as against 27.0° and 30.5° on January 1), and although there had probably been no thawing whatever during January, the sap densities of both spruce and bristlecone pine decreased during the month. The evaporation rate from February 1 to 6 was only about one-fifth as great as from December 26 to January 1, meaning, of course, much less current desiccation in the later period. But it is also indicated that at some time between January 1 and February 6 the leaves must have obtained moisture from some source. This might have been by transfer from the stems, if thawing of aerial parts occurred; but there is no apparent reason why the leaves should not have taken up vapor from the atmosphere during periods when the vapor of the atmosphere was practically saturated. The possibilities of such absorption, as a relief for winter drought conditions, are too important to be overlooked.

The material collected May 7 showed in all cases much lower sap density in the twigs than in the more exposed half of the needles, the latter being selected because logically subject to the greatest drying. This indicates that small variations in the results may be expected from clipping slightly more or less than half of the needles.

All specimens showed a decided drop in densities on May 7, by which time there was a great deal of surface thawing, except, as explained, around the roots of the spruce at Station F–16. This tree responded in the next period.

The spruce and alpine fir obtained from a protected stand in a gulch near F–13 both on February 6 and May 18 show plainly the advantages

of protection. The high density of the last spruce in the test, on May 18, is difficult to explain, in view of the exposure on a southwesterly bank and the apparent abundance of moisture. This may be due, however, both to the full exposure of the tree to light and to a possible high degree of nonavailability of the moisture as a result of acidity and lack of aeration.

Summarizing, it is evident that the sap density of any species or individual is not a stable quality but varies according to the amount of photosynthesis which is permitted and according to current conditions affecting water supply and transpiration. Nevertheless, there is found in these data no reason for changing the original conclusion that, given equal opportunities and exposures, the sap density of spruce will be higher than that of any of the other species; that spruce will tolerate a very great loss of moisture, and a resultant high sap density in the needles, without injury; and that it can, therefore, be said that spruce is not only better equipped to resist transpiration, other things being equal, but that the ability to resist transpiration and its possibly injurious effects makes spruce in reality the least moisture-demanding of all of the Central Rocky Mountain trees.

These conclusions, however tentative, must at least develop a wariness to accept average or temporary moisture conditions of the site as prima facie evidence of the relative moisture requirements of the species occupying it.

On the other hand, we have as yet no evidence that spruce is more drought-resistant than other species or that the sap density and the specific qualities that affect it react upon distribution through the water requirements. All that we have so far been able to show is that low sap density permits a species to occupy warm sites where the exposure is very great most of the time, while high sap density appears to hold the species to cool sites, where the winter drought may be severe, especially at high altitudes.

WILTING COEFFICIENTS FOR DIFFERENT SOILS AND SPECIES

If it could be shown that one species is capable of extracting the moisture of the soil to a lower point than other species before wilting or other injury to the plant was apparent, this would constitute direct evidence that the first species not only was less likely to experience fatal drought conditions but also was capable of sustaining higher internal osmotic pressures without injury to the protoplasm. When wilting occurs, if the condition has been approached gradually, it may be assumed that the osmotic pressure in the plant is essentially the same as in the soil, and the latter, of course, increases as the moisture content decreases and the concentration of the soil solution increases.

It has already been indicated in connection with transpiration in 1920 that spruce appeared to be able, under all conditions, to obtain the water required for free transpiration more nearly than any of the pines or Douglas fir. A similar test with the moisture gradually reduced to complete nonavailability would, perhaps, be preferable to wilting tests, which must be conducted with seedlings in order that the end-point may be observed ocularly. It is fairly evident that the seedlings may not show development of the internal characters which are important in this connection to the same extent as older trees. However, it can not be gainsaid that it is the seedlings which are subjected to the greatest dan-

gers, and it is their performance, rather than that of older trees, which determines the composition of forest types.

Although only a few wilting tests have been made in which the several species have been observed growing in the same soil, a considerable amount of information has been obtained on each species in a variety of soils; and by reference to the physical properties of these soils we may obtain fairly satisfactory comparisons. In each case, the species used was that one which occupied the given soil or predominated in the type in the field.

PROCEDURE

In general the intention has been to secure the wilting coefficient for the soil as found in the field—that is, with the normal admixture of rocks and gravel, since the saturation and capillary capacities and other physical measurements were on this basis. In this respect mountain soils present difficulties ordinarily not met with in agricultural work.

To attain this end it is not sufficient to sample the soil for moisture content after the seedlings have wilted. The mositure content must be determined for a mass of soil large enough to represent normal proportions of rock and finer material.

Pans about 10 inches square were used in the earliest tests, the soil being in a layer from 1 to 1½ inches deep. These were sometimes found to be too shallow to accommodate the rocks which should be included; consequently a standard round pan was specially made, having a diameter of 7 inches, a depth of 3 inches, a soil depth of 2½ inches, and an ordinary soil weight of about 4 pounds. A few holes were punched in the bottom of each pan to prevent excessive wetness and to aid aeration, it being the belief that with the pans on a bench the evaporation rate through these small holes could never be an important factor. In fact, though the soil surfaces have usually been paraffined, it has never been attempted to make the coatings air-tight, since the object is not to prevent water loss from the soils but to insure that when wilting occurs the moisture distribution throughout the soil shall be fairly uniform.

The vegetation has been secured by sowing seeds of the desired species in the pan of soil, watering these moderately, and permitting the seedlings to develop for about a month before coating the pans and cutting off the moisture supply. Beyond the age of a month the seedlings may rapidly lignify, so that the wilting does not occur promptly or is very difficult to detect. This is true of Douglas fir seedlings at any age. It is, of course, realized that seedlings of this age may not exercise the same control over moisture as would older trees.

The soil sample is placed in the pan in an air-dry condition and is oven-dried to determine its net weight. This practice may have had some effect on the colloids but is fully justified by the assurance it gives that micro-organisms will be eliminated and will not cause the untimely death of the seedlings. However, as mountain soils are rarely strong in clay, as the samples have always been air-dried first, and as the oven temperature has been only 92° C., it is thought any change in soil qualities may virtually be ignored.

After drying, a cupful of soil is taken from the pan, a weighed lot of seeds is strewn over the smooth surface of the remaining soil, and the cupful is then used to cover the seeds.

The moisture applied to induce germination and development of the seedlings has usually been left wholly to judgment, the intention being to give all that can be used and never to permit the surface to become dry.

The seedlings have been developed with abundant sunlight, in the greenhouse, avoiding excessive temperatures as far as possible. To make a satisfactory test each pan should develop at least 100 seedlings.

When the final weight of a pan is secured, with the seedlings wilted, deductions are made for the known weight of paraffin applied, as also for the weight of the seed used, which is assumed to be the same as that of the wilted seedlings and the loose hulls. This weight could generally be ignored without affecting the result appreciably, for the moisture content is usually 60 or 80 gm., as against 1 to 5 gm. for the seed of any species except yellow pine.

DIRECT COMPARISONS OF THE SPECIES

Not until 1920 was it possible to conduct special tests with two or more species in the same soil. The most comprehensive test, and therefore the least likely to be misleading, was conducted from April to September, 1920. In this case the soils were not sterilized by oven-drying, and considerable damping off of the seedlings occurred, which may be confused with legitimate wilting in the early stages. The pans were watered and weighed daily, and the losses of seedlings were recorded, so that it is possible to consider the losses at any stage. Because of the damping off, and also to make these results more comparable with those in which but one wilting period was recorded—that is, the time when practically the entire number collapsed—it seems best to consider in all these more recent tests the mean wilting point for the last 25 per cent of the total number of seedlings observed. Not infrequently the weakest seedlings die with twice as much water available as is required to sustain the strongest.

In this particular test the moisture equivalents of the five soils were determined first, under a force of 100 gravity; and, assuming that these quantities were indicative of the same degree of availability in each soil, the watering in each case was so gauged as to maintain this moisture equivalent. It may be remarked that this quantity was very favorable for germination and establishment. Later the water content of each pan was reduced to two-thirds of the moisture equivalent, finally to one-third, and from that point downward by 5 gm. stages. This is important because other tests indicate that the drought which a seedling will tolerate depends much on the moisture to which it has become accustomed.

The soils in this case were not paraffined, but some water was given almost every day in order to eliminate, so far as possible, extreme drying-out of the surface. It is significant that, perhaps on this account, the wilting coefficients are relatively lower than usual.

The results of this test, which have already been given in the research manual (*4, pt. 1*) to illustrate the relation between wilting coefficient and moisture equivalent, are given in slightly different form in Table XIX.

We shall not discuss the rather variable relations of these wilting coefficients to the physical measures of moisture-holding properties of the soils. Suffice it to say that other evidence points to the fact that the several wilting coefficients represent an osmotic constant in the different soils, while either the capillary moisture or moisture equivalents fall considerably short of this. This relationship will be discussed in connection with the field-moisture problem. The physical measures of soil moisture bear only a general relation to wilting coefficients and must be used with this understanding.[6]

[6] The reader is urged to note the discussion of this by Bates and Zon (*4*) where it is made plain that a constant ratio between wilting coefficient and moisture equivalent is impossible if a wide variety of soil types is considered.

The important thing shown by Table XIX is that the wilting coefficients of yellow pine, Douglas fir, and Engelmann spruce are essentially the same in all the soils, while that for lodgepole pine is much higher. There is, moreover, no evident reason for the fact that in some of the soils (sandstone and prairie shale) the wilting coefficient for spruce is lower than for either pine or Douglas fir. We must, at least at this stage, regard these variations as accidental.[7]

It will now be well worth while to determine whether, as between any two of these apparently equal species, a greater number of results brings out any difference. A very considerable amount of data has been secured on Douglas fir and spruce growing in the same soils. In introducing these data it is desirable to point out:

1. That ocular observations on wilting, especially when the moisture supply is steadily declining, tend to favor Douglas fir, because that species has a much stronger and more fibrous stem and rarely collapses. The evidence of wilting is, therefore, much less plain than in the frail spruce seedling, and, it seems likely, may not be obtained until a day or two after the fatal condition has first existed.

TABLE XIX.—*Wilting coefficients of the four important species in 5 types of soil*

Kind of soil.	Capillary moisture.	Moisture equivalent 100 gravity.	Mean wilting coefficients, best 25 per cent of the seedlings.				
			Yellow pine.	Lodgepole pine.	Douglas fir.	Engelmann spruce.	Average of four.
Granitic gravel sandy loam	*Per cent.* 26.58	*Per cent.* 10.55	*Per cent.* 2.28	*Per cent.* 2.42	*Per cent.* 2.16	*Per cent.* 2.26	*Per cent.* 2.28
Composite limestone loam	31.85	22.00	3.23	4.12	3.56	3.50	3.60
Composite sandstone loam	35.34	21.77	4.10	5.29	4.30	3.93	4.40
Prairie silt loam from shale	37.77	28.79	7.80	8.69	7.79	7.43	7.93
Composite lava silt loam	43.16	27.80	4.52	6.00	4.97	4.87	5.09
Average			4.39	5.30	4.56	4.40	4.66

2. That in the early stages of development Douglas fir roots more strongly than spruce and its roots reach a greater soil area, but particularly in these pan tests they reach the deepest layer of soil which may not be drawn on at all by the spruce if the wilting is accomplished at an early age. (See Plate 7, B.)

3. Therefore, in these tests it is evident that if spruce seedlings tolerate as great a degree of drought as fir, the moisture being known only through the whole pan weight, it must be through greater ability to extract water from the soil.

From Table XX it will be evident that the wilting coefficient for spruce is, on the whole, higher than that for Douglas fir. The difference is only about 3 per cent of the value for fir. Of the 23 cases cited, only 6 give spruce a lower value than fir, and 5 of these 6 are among the loose gravels

[7] Since the foregoing statement was written very convincing results have become available showing the different effects of each of these soils in stimulating the growth of each species, both according to chemical composition of the soil and the concentration of its solution. It can hardly be questioned that this has a direct bearing on the behavior of the seedlings as the wilting point is approached, and in fact that this entire problem is quite as much one of chemical relations as of the physical relations which have been discussed in this paper. It is hoped that something may be published on this chemical phase in the near future.

or sands of granitic origin, while only 1 is found in the more loamy soils. This is at least suggestive that in the soils of freer capillary movement the very meager root system of spruce is not so great a disadvantage. From these facts we certainly can not draw the conclusion that in the osmotic sense spruce has any less control over soil moisture than fir.

INDIRECT COMPARISONS OF THE SPECIES

A very considerable amount of information has been secured in the somewhat routine process of determining the wilting coefficients for a large number of soils of almost every possible origin in connection with nearly every study in which soil quality or soil moisture has been an important factor. For the most part the wilting coefficients have been determined for each soil only with respect to one species, that one being the species which characterized the soil or site in the field. It is obviously necessary, before these results may be used for a comparison of the species, that each result should be related to some other measure of the moisture-holding properties of the soils, and the best measure at present available for any considerable number of the soils is the moisture equivalent at 100 gravity. As we have seen in Table XX, however, even this does not bear a constant relation to wilting coefficients, when radically different types of soil are considered. Particularly does it seem that the coarse-grained granitic soils of the Pikes Peak region, which we have studied more than any other, have an unusually weak hold on the water until the amount is brought close to the wilting coefficient, so that the moisture equivalents of these soils are relatively low.

TABLE XX.—*Comparative wilting coefficients of spruce and Douglas fir in the same soils*

Kind of soil.	Moisture equivalent.	Wilting coefficient.		Ratio of wilting coefficient to moisture equivalent.	
		Fir.	Spruce.	Fir.	Spruce.
	Per cent.	*Per cent.*	*Per cent.*		
Granitic gravels over 50 per cent rocks and coarse gravel, less than 20 per cent silt and clay.	3.53	1.30	1.39	0.368	0.394
	4.35	1.73	1.94	.398	.446
	4.86	2.05	2.03	.422	.418
	5.03	1.77	1.80	.352	.358
	5.04	2.14	2.23	.424	.442
	5.06	2.54	2.34	.502	.462
	5.19	2.01	1.87	.387	.360
	5.57	1.94	1.71	.348	.307
	5.62	2.13	2.61	.379	.464
Granitic sandy loam	11.05	2.94	2.91	.266	.263
Granitic spruce soils, varying from rocky coarse sand to silt loam.	11.68	2.72	2.88	.233	.246
	11.72	2.53	2.79	.216	.238
	14.45	3.42	4.09	.239	.283
	19.95	6.41	6.44	.321	.323
	20.32	5.60	5.78	.276	.284
	22.02	5.25	5.61	.238	.255
	26.30	8.34	8.17	.317	.311
	29.84	7.44	7.98	.249	.268
	42.72	17.09	17.40	.400	.407
	73.50	17.53	18.56	.238	.253
Quartz latite rocky sandy loams	11.96	5.13	5.20	.429	.435
	13.98	6.96	7.14	.498	.510
	14.85	5.72	6.04	.385	.407
Average of all				.3427	.3537
Mean difference					.0110
Probable error in mean difference					.0029

TABLE XXI.—*Miscellaneous wilting coefficients.*

YELLOW PINE

Sample No.	Station or forest.	Origin and character of soil.	Moisture equivalent.	Wilting coefficient.	Ratio of wilting coefficient to moisture equivalent.	Conditions of test.
			Per cent.	*Per cent.*		
326	Nebraska	Aeolian ridge sand	3.72	0.54	0.145	Without paraffin.
325	do	Aeolian bottom very fine sand	12.36	2.51	.203	Do.
70	Fremont F-2	Granite gravel	5.41	1.16	.214	
71	do	do	5.91	2.63	.445	
72	do	do	5.86	2.67	.456	
73	do	Granits sand	9.29	3.67	.395	
78	Fremont F-4	Granite gravel	5.20	1.79	.344	
79	do	do	4.69	1.28	.273	
80	do	do	3.70	1.43	.387	
26	Fremont F-6	do	6.73	2.44	.363	
29	do	do	5.24	2.65	.506	
25	do	do	4.48	1.85	.413	
121	Fremont F-12	do	5.87	1.99	.339	
132	do	do	4.62	1.97	.427	
130	Pike M-1	Granite coarse sand	8.91	2.36	.265	Do.
124	do	do	6.61	1.53	.232	Do.
117	do	do	6.76	1.68	.249	Do.
53	Black Hills	Limestone silt loam	30.55	15.33	.502	Do.
55	do	Sandstone loam	16.02	2.58	.161	Do.
62	do	Sandstone silt loam	22.30	6.41	.287	Do.
63	do	Schist loam	19.06	6.26	.328	Do.
122	Colorado	Sandstone fine sandy loam	16.96	9.97	.587	
127	Wagon wheel Gap.	Quartz latite loam	21.95	3.77	.172	
101	Cache	Volcanic ash, etc	28.23	12.57	.445	Do.
102	do	Silt loam	28.05	9.89	.342	Do.
103	do	do	29.66	10.14	.342	Do.
104	do	do	27.27	10.40	.382	Do.
632	Wagon wheel Gap.	Quartz latite sandy loam	18.43	7.50	.407	
	Fremont	Granitic sandy loam	10.42	4.09	.392	

LODGEPOLE PINE

1	Arapaho	Granitic loam	17.03	7.34	0.431	
2	do	Granitic sandy loam	11.32	3.02	.267	
3	do	Granitic fine sandy loam	16.75	3.50	.209	
4	do	do	7.63	2.75	.360	
5	do	Granitic coarse sand	7.75	5.70	.736	
6	do	Transported fine sandy loam	13.65	2.96	.217	
262	Medicine Bow	Gneiss coarse sand	13.45	4.72	.351	Without paraffin.
263	do	do	16.25	3.65	.224	Do.
264	do	do	11.62	2.99	.257	Do.
265	do	do	7.62	.76	.100	Do.
533	do	Gneiss fine sandy loam	12.90	4.69	.364	
540	do	do	13.78	5.22	.379	
541	do	do	14.75	4.75	.322	
538	do	do	13.76	5.61	.408	
559	do	Gneiss fine sand	10.22	4.00	.399	
127	Wagon wheel Gap.	Quartz latite loam	21.95	4.41	.201	
71	Leadville	Sandstone silt loam	21.34	1.90	.089	Do.
12	do	Sandstone fine sandy loam	12.35	2.52	.204	
57	Fremont F-10	Granitic sandy loam	9.27	3.55	.383	
48	Colorado	do	10.39	3.34	.322	

DOUGLAS FIR

8	Arapaho	Igneous fine sandy loam	13.31	4.12	0.309	
66	Fremont F-1	Granite gravel	4.29	2.31	.538	
67	do	do	3.60	.93	.258	
68	do	do	4.34	.76	.175	
69	do	Granite sand	8.25	2.13	.258	
17	Fremont F-7	Granite gravel	4.71	1.26	.268	
19	do	do	4.94	1.24	.251	
18	Fremont F-9	do	5.03	.96	.191	
127	Wagon Wheel Gap.	Quartz latite loam	21.95	5.11	.233	Without paraffin.
533	Medicine Bow	Gneiss fine sandy loam	12.90	4.04	.313	
540	do	do	13.78	4.88	.354	
541	do	do	14.75	4.44	.301	
538	do	do	13.76	5.50	.400	
559	do	Gneiss fine sand	10.22	3.76	.368	

TABLE XXI.—*Miscellaneous wilting coefficients*—Continued

ENGELMANN SPRUCE

Sample No.	Station or forest.	Origin and character of soil.	Moisture equivalent.	Wilting coefficient.	Ratio of wilting coefficient to moisture equivalent.	Conditions of test.
74	Fremont F-3	Granitic gravel	7.87	1.54	0.196	Without paraffin.
75	do	do	6.53	1.51	.231	Do.
76	do	do	3.99	1.50	.364	Do.
76	do	do	3.99	1.40	.351	With paraffin.
35	Fremont F-5	do	6.74	2.12	.315	Without paraffin.
33	do	do	7.62	2.64	.347	Do.
34	do	do	6.68	1.98	.297	Do.
110	Wagon Wheel Gap D.	Quartz latite sandy loam	20.50	7.56	.369	
111	do	do	17.38	3.59	.207	
112	do	do	17.72	4.14	.234	
632	Wagon Wheel Gap A-1.	do	18.43	6.93	.376	
	Fremont	Granitic sandy loam	10.42	3.73	.358	
6	Arapaho	Transported fine sandy loam	13.65	3.22	.236	
243	Leadville	Granitic loam	45.40	7.60	.168	Do.
231	Battlement	Lava loam	30.16	4.93	.163	Do.
232	do	do	21.83	3.73	.171	Do.
205	Bighorn	Granitic sandy loam	17.40	2.88	.166	Do.
206	do	do	13.63	2.17	.159	Do.
222	Gunnison	Limestone silt loam	28.16	3.31	.118	Do.
221	do	Limestone loam	22.78	2.69	.118	Do.

In Table XXI there are presented all the wilting coefficients which have not been given in the two preceding tables, and for which the corresponding moisture equivalents are available. On examining the data, however, it is readily seen that in each group those tests which have been made without coating the soil with paraffin give much lower coefficients than those in which the soil was coated. This is as might be expected and, since the proportion of such tests is variable in the different groups, it seems best to discard all data obtained in this way. We then obtain the following averages:

Species.	Number of tests.	Mean moisture equivalent.	Mean ratio wilting coefficient to moisture equivalent.
		Per cent.	
Yellow pine	16	8.42	0.3825
Lodgepole pine	15	12.90	.3468
Douglas fir	13	8.76	.3065
Engelmann spruce	7	14.58	.3044

When allowance has been made for the fact, which is evidenced by the mean moisture equivalents, that three-fourths of the usable data for yellow pine and half of that for Douglas fir were obtained with granitic gravel, or sand soils, although these soils do not much effect the other two groups, it is definitely decided that wilting coefficients are much lower for Douglas fir than for yellow pine, probably somewhat lower for yellow pine than for lodgepole, and certainly lower for Douglas fir than for spruce.

With this confirmatory evidence, no hesitancy need be felt in placing these four species in the following approximate relationships:

Species.	Approximate mean ratio of wilting coefficients to moisture equivalents on common basis of soil qualities.	Species.	Approximate mean ratio of wilting coefficients to moisture equivalents on common basis of soil qualities.
Lodgepole pine	0.35	Engelmann spruce	0.32
Yellow pine	.33	Douglas fir	.31

Certainly, from all the evidence available, the differences between the species are not any greater than here indicated. From all that we have found, it would probably be fair to say that in actual ability to stand drought, at least for the conditions existing in these pan tests, there is no essential difference between yellow pine, Engelmann spruce, and Douglas fir, the greater frailty and slow rooting of the spruce as compared with fir or pine being balanced by an actually stronger affinity of the spruce for any water within reach of its roots. On the other hand, lodgepole seems to stand out both as frail and slow-rooting, and with no compensating development of high sap density or osmotic pressure, so that it does succumb to drought much sooner than the others. In one test only, limber pine and bristlecone pine have shown themselves in practically the same class as yellow pine.

RESISTANCE TO EXCESSIVE HEAT

The sap densities observed in seedlings, and the relative rates of transpiration as apparently affected thereby, gave rise to the suggestion that there might be a specific difference in heat requirements based on this same set of internal conditions. While, on the one hand, the freely transpiring species of low sap density would seem to require a warm environment to counteract the cooling effect of this transpiration, on the other hand, the species of high sap density, which also seem to function more fully than others without full direct sunlight, appear to be always in danger of becoming overheated because, for some physical reason not fully explained, the heat absorbed is not so fully utilized in evaporation.

One thing which the close observation of seedlings in the wilting tests has made very plain is that at an early age all seedlings are very susceptible to injury just where the stems are in contact with the surface soil. At times it has seemed as though moisture absorbed by the roots might be extracted from the stems at this point, so blanched and shrunken do they become as soon as the surface of the soil becomes dry. On the other hand, it is perfectly evident that, as soon as the surface soil ceases to possess moisture to keep its temperature down, it may in sunlight easily become by far the warmest part of the environment. The measurement of the temperature at the warmest point is exceedingly difficult, but the showing of thermometers more or less submerged indicates that the soil surface not infrequently attains a temperature

of 160° F. It is, therefore, readily seen that in soils exposed to sunlight the injury resulting from drought at the surface may be indistinguishable from that due to superheating. Under ordinary circumstances the two injurious conditions will be inseparable.

The difficulty of determining the heat tolerance of seedlings at the point where they are commonly injured by heat is, because of the influence of moisture, very great. We have not been able to conceive a test of heat tolerance in the normal sense, except through the employment of sunlight or some other more powerful radiant energy. Every other possible plan of exposure to heat seems to have the objectionable result of injuring the foliage first, which rarely happens in nature, or of preventing normal evaporation with whatever protection that may afford.

Therefore, the only test [8] that has been made to determine the relative tolerance of heat by forest-tree seedlings has been on this basis of obtaining as high temperatures as possible in sunlight, with the air to some extent artificially warmed. The actual temperatures attained were measured only so far as was possible by placing mercurial thermometers directly above the soil surface. The seedlings of each species were developed in several pans, each of which represented a different moisture content. Because of the fact that the largest amounts of soil moisture permitted almost no injury, the moisture contents were in several cases lowered before the test was completed, so that the record is considerably confused. From the data secured, however, the following conclusions, admittedly tentative, may be drawn:

1. Injury to seedlings from excessive heat is plainly greatest when the seedlings are youngest. This introduces a complicating factor in the test, because exposures to high temperatures were begun before germination was entirely completed and when, therefore, there was the most marked difference in ages. Engelmann spruce ordinarily germinates most promptly and spontaneously. Consequently, while there was marked early damage to this species, the fact that there were few later germinations left the species then immune for some time. Lodgepole pine exhibits just the opposite characteristics and effects.

2. Seedlings which survive a certain degree of exposure are not likely to be injured until the conditions become considerably more severe.

3. The ease with which any species may be injured increases very markedly as the moisture content of the soil decreases. With the lowest content, 3 per cent, which in this soil was appreciably above the wilting coefficient, it may be questioned whether the injury was not due to drought almost wholly, since between waterings the wilting coefficient of the soil was reached.

4. At all times the nature of the wilting was indistinguishable from that which occurs with simliar seedlings when no excessive heat is involved. Consequently, it appears that wilting may be due as much to inability to supply transpiration losses as to the direct effects of the temperatures. The fact that no wilting was secured with 14 per cent moisture appears to bear out this idea, yet it must be remembered that this free moisture may have greatly reduced the temperature extremes of the surface soil. The fact that temperatures recorded just above the soil were not in excess of 135° F. further suggests that wilting was the result of transpiration losses rather than a direct temperature effect on the protoplasm.

[8] Credit for the conduct of this test should be given to Forest Asisstant J. Roeser, jr.

5. With this understanding of the situation we may say that in this test Engelmann spruce and lodgepole pine were most susceptible, while Douglas fir and yellow pine were about equally resistant. The factor which seems to control susceptibility is mainly structural rather than physiological—that is, it is the small mass of the spruce and lodgepole, and possibly their weak rooting, which causes them to stand out in contrast to yellow pine and Douglas fir under extreme drying conditions of relatively short duration.

This pairing of spruce with lodgepole suggests as strongly as do the high wilting coefficients for lodgepole the very poor ability of the latter to supply itself with water; but, in the light of the other facts secured, the same cause will not fully explain the behavior of spruce. It is believed it would be fairer in the case of spruce to say that high temperatures in direct sunlight create high internal temperatures and some direct heat injury. This hair-splitting distinction is necessary for the proper physiological interpretation which will agree with the other facts at hand. It may be added that the susceptibility of spruce to injury in sunlight has been very evident in many of the wilting coefficient tests.

EVIDENCE OF WINTERKILLING

Winterkilling of trees is generally recognized as the direct result of evaporation from the leaves or twigs at times when moisture can not be supplied to replace the loss, owing to a frozen condition of the soil. It is, of course, not confined to evergreen trees but may affect fruit trees, or even such hardy forest trees as honey locust, when devoid of foliage.

The conditions for winterkilling are usually provided by a very rapid rise in air temperatures and by wind which facilitates evaporation. The soil, of course, warming more slowly than the air, may not free its moisture for many hours after the beginning of the unseasonable air conditions. Likewise, if the tree stems have been thoroughly frozen, they may not be able to transport water until a great loss from the leaves and small twigs has occurred.

The conditions conducive to winterkilling are especially likely to be produced near the base of the Rockies from northern Colorado northward. The coniferous forests which are subject to this form of injury are therefore the low-lying yellow pine forests of the Black Hills and eastern Montana. Here the Chinook, a warm wind occurring at a season when the normal temperatures are below freezing, attains its most typical development.

A typical Chinook has not been noted within the locality of the present study. It has been shown, however, that in the Pikes Peak region the winds from January to March possess the powers of a modified Chinook. While they do not often bring extremely large rises in air temperatures, they are of high velocity, the air is dry, and the soils at all elevations, unless strongly isolated, are likely to be deeply frozen and remain so throughout the duration of the wind, which is often two or three days.

The Pikes Peak region therefore presents a good opportunity for the study of the relative resistance of the several species to this form of drought, for the desiccating influence is not confined to the low zone where only yellow pine occurs.

The present writer (*2*) has described in some detail the cumulative effects of winds occurring at the Fremont Station in January and March, 1916. It was shown that on a south exposure where yellow pine, Douglas

fir, and limber pine grow in a mixed stand, Douglas fir at first showed a more pronounced discoloration, but later the injury to yellow pine was seen to be much more severe, as only this species was defoliated. This injury was always much more pronounced on the west (windward) side of a tree, but it varied with different specimens, partly because the ground is strewn with large boulders which deflect the wind and also reflect sunlight. While in no case fatal (and even the general injury in the Black Hills in 1909 caused a very small percentage of deaths), this defoliation obviously must have a retarding effect on the growth of the whole tree. That the same kind of injury occurs at intervals of a few years, and that it hits "twice in the same spots," seems to be indicated by the one-sided development of most of the trees which were injured in 1916 (see Pl. 7, A). Buds and branches were rarely injured in this case, and new foliage appeared almost as early as on unaffected trees or parts.

In the nursery, where there was no snow to furnish protection during most of the winter, a better comparison of the species was possible because of the uniform conditions of soil and exposure. Yellow pine stock was damaged more than Douglas fir; Douglas fir far more than spruce. In fact, in only a few cases was spruce even discolored. With lodgepole the injury was usually confined to an exposed branch or leader, suggesting incomplete ripening of the previous season's growth.

This indicates, as do all other data, that spruce can bear drying to a greater degree than the other species, or at least that it resists the drying better, which comes to the same result. It is perhaps significant of the moisture-conserving adaptation of limber pine, which has been indicated by the transpiration tests, that there was no apparent injury to this species on the south slope where yellow pine was most plainly injured. It resisted wind-drying of this kind as well as any species. On the other hand, during the summer drought of 1917, limber pine was the only species showing injury to trees of large size.

SUMMARY

The relative qualities of the important forest trees of the Central Rocky Mountains, primarily from the standpoint of moisture relations, have been approached from five different angles. No one of these efforts has been free from errors, and no one would alone carry conviction, but the several results are corroborative with only insignificant exceptions. These comparisons of the species have been made on the basis of—

1. Measurements of the water used in relation to growth and leaf exposure of 3- to 9-year-old trees, under uniform conditions for all species.
2. Comparisons of sap density under uniform and varying growth conditions.
3. Measurements of the moisture of soils not available to young seedlings by direct comparisons of the species and also under varying conditions as to soil quality and atmospheric stresses.
4. Observations on fatality among seedlings under high temperature conditions.
5. Observations on the resistance to winter drought of specimens growing side by side, and as measured by the extent of injury to foliage.

It will have become apparent that there are several aspects of the moisture relations, that the several species studied do not always stand in the same relation one to the other, and that it is not even possible to state

that of the Rocky Mountain species one is distinctly more drought-resistant than the others. The mositure relations apparently vary much with the other environmental conditions, and it is perhaps the most important feature of this paper that a somewhat logical relationship has been shown to exist between moisture requirements and other requirements of each species.

We may, then, briefly outline the theory and at the same time observe how closely it applies to the behavior of each species under each of the situations that has been presented. We may take as our starting point the relative "tolerance to shade" of the several species, because this is a character which always has been quite closely observed by foresters and in which, empirically, rather definite lines have been drawn.

Briefly the physiological requirements appear to be related on this basis:

1. The species of greatest shade tolerance or greatest ability to make effective use of sunlight in photosynthesis will possess, other conditions remaining equal, after a period permitting accumulation the greatest amount of soluble carbohydrates in the leaves. In this fundamental respect we shall adhere, at least tentatively, to the classification indicated by the December, 1917, sap densities, as shown in Table XIII, placing spruce at the head of the list of our indigenous species, followed by Douglas fir, lodgepole, bristlecone, yellow, and limber pines.

2. The presence of considerable quantities of carbohydrates augmenting other solutes creates a dense sap, or solution, which does not evaporate so readily as a dilute solution. Because of the osmotic pressure exerted by a dense solution, there should at the same time be greater ability to extract water from the soil, though there is no evidence that at the end of the struggle one species tolerates appreciably greater drought than the others.

3. The presence of these solutes in large quantities is also, naturally, conducive to a high growth rate.

4. By restricting evaporation, the soluble carbohydrates may increase the net amount of the light energy available for photosynthesis, so that, whatever the original quality which made the plant effective, this quality is augmented by its own results.

5. By restricting the use of heat in evaporation, however, the dense cell sap may not only reduce the relative heat or light requirement of the species but may subject it to the danger of superheating. Of all the possible influences of the specific differences which give rise to the cell-sap differences, it is believed this is the most important ecologically and the most potent in its effect on the distribution of the species. If we assume distribution to be controlled primarily by this physiological factor, it becomes fairly simple to see how adjustments have been made to meet other conditions of the environment, principally in the form of structural adaptations, which differentiate the species beyond that difference which may arise from photosynthetic efficiency, and which may to a certain extent compensate for the physiological deficiencies.

If we accept the heat hypothesis as fundamental, we mean that each species will be limited in its distribution rather sharply by the maximum temperatures which it can tolerate (probably in the early seedling stage) and also limited in its growth by its minimum requirements, so that at a certain low temperature it is unable to compete with more highly developed species and hence loses its dominance in the forest. In the mountain forests, therefore, we should expect to find the six species zonated according to temperatures, in the order named just above.

This zonation holds, definitely, however, only for Engelmann spruce, Douglas fir, and yellow pine, which we have shown to be so equally developed as forest dominants that the fundamental physiological differences control all their relations. With the three more or less weedy pines there are, plainly, adaptations which are equally effective or more effective in controlling distribution. It is significant of the importance of high temperatures as absolute limitations that these three species are all found in higher and cooler zones than their physiological conditions necessitate.

Supplementing physiological characters, we may have stomatal reduction, thickened epidermis, or clustered leaves, all tending to reduce the absolute transpiration, but, while doing so, inevitably reducing either the intake of carbon dioxid or the effectiveness of sunlight so that photosynthesis and growth are reduced perhaps even more than is water loss. This seems to be the general line of protective development in the "weed" trees, limber pine and bristlecone pine, and to a lesser extent in lodgepole pine.

Again, resulting from gradual adjustment to the moisture conditions which accompany certain heat conditions, the forest trees have different root habits, or (shall we say?) are unequally stimulated to root development. It is believed that temporary stimulus has much to do with it, but inherited habit still more. Be that as it may, yellow pine and Douglas fir root much more vigorously at an early age than lodgepole pine or spruce. Almost as divergent are the germinating rates of the seed, lodgepole pine standing out as the most sluggish of the six species studied.

In the strictly physiological sense, spruce is undoubtedly the most highly developed of the indigenous species we have considered. This is evidenced by the sap densities which the trees show after long seasons of photosynthesis and by the amount of growth made in relation to the total amount of the water consumed.

In actual water consumed by a tree exposing a unit area to light (and wind) spruce is again the most economical, followed by Douglas fir, bristlecone, limber, yellow, and lodgepole pines. In this consideration, it is, almost without question, the special adaptations of the weed pines which put them down as only moderately extravagant, making them especially suited to exposed windy sites but wholly incapable of holding a permanent place in the forest. On the contrary, spruce maintains a moderate rate of transpiration under the driest conditions (so far as measured) for two reasons, namely, because it does not mechanically restrict losses but forges ahead with growth, and because when the water supply is low it is still more able than any of its competitors to supply its needs and is not so soon restricted either in transpiration or growth. These facts stand out very clearly. In this comparison we have placed Douglas fir next to spruce, believing that the actual position shown by Table XI is misleading, because the trees involved did not develop normally.

In resistance to winter-drying, limber pine with its peculiar structural development and spruce with its high physiological resistance have shown themselves about equally effective. Douglas fir and yellow pine follow with increasing weakness. Lodgepole pine shows greater resistance than would be expected, a fact which we shall not attempt to explain at present.

Considering the drought resistance of seedlings, through the wilting coefficients of a number of soils in which they have been compared, we find the same physiological properties evidently at work, though much

obscured by the relative sizes of the seedlings and their root developments. The seedlings of spruce and lodgepole pine are small and frail and in the first two or three months develop scarcely more than half the root produced by Douglas fir and yellow pine. As a result, even when carefully protected from excessively rapid water loss, lodgepole pine seedlings show far less drought-resistance than the others. Spruce seedlings, on the other hand, show quite as great resistance as those of pine or fir when not excessively insolated, and possibly a little more if the drought condition is approached every slowly. Limber and bristlecone pine seedlings, as meagerly observed, resist drought with the best of the others, no doubt because of a low rate of transpiration. In this connection the soil conditions leading up to wilting of seedlings should be borne in mind. Rarely is it possible for the roots to reach and extract all of the moisture which it would be physically possible for them to absorb. The completeness of this absorption depends very much on capillary movement in the soil. If the amount required by the seedling is small, this movement may supply the needs. Therefore, the rate of transpiration by the seedling is very important in determining, to a fine point, the degree of drought which it will resist.

In nature all possible rates of soil-drying are represented, dependent very much on the amount of insolation on the site and to some extent on the nature of the soil cover. The open south exposure will usually dry at the immediate surface very rapidly. Because of the lack of humus, however, the layer just below the surface may remain moderately moist so long as the quantity of water below is sufficient to maintain capillary movement. When that end is reached, the soil undoubtedly dries out very rapidly to a considerable depth. Even with the respite furnished by capillary movement, the whole process of drying, in continuously bright and dry weather, seems likely to be accomplished here sooner than in the contrasting site. This may be on bottoms or north exposures where the total moisture supply is sufficient to produce a closed stand, heavy shade, and the accumulation of humus. In this soil the surface litter and humus are rarely thoroughly wetted except during and immediately after the melting of snow. The more decomposed humus below, however, due to a lack of insolation and being protected by the surface litter, is rarely dry except after prolonged drought. It dries out slowly and steadily, however, both through the demands of the roots below and by direct evaporation. It follows that, since these demands in the aggregate are very large, such a soil may at unusual times, or possibly in the usual autumn drought, become extremely dry, especially so in the physiological sense, because of its high wilting coefficient.

On the one hand, then, we have the rapidly fluctuating moisture conditions of the well-insolated site, which, for the establishment of seedlings would appear to demand prompt germination and prompt deep rooting. Yellow pine is preeminently adapted to these conditions by reason of its large seeds, which produce large sturdy seedlings with a habit of immediate deep rooting. There is nothing in the evidence on this species to suggest conservatism in the use of water. Probably the extravagant use of water assists in protecting from excessive heat. Success is dependent on the roots reaching a layer of the soil which does not dry out dangerously through insolation. It follows logically that yellow pine can not attain success in the face of competition, either with the roots of established trees or with grasses and herbs whose use of the water would materially augment the drying of the surface layer.

The large moisture demands of yellow pine, with the normal amount of precipitation, can only be supplied in open stands, which first permit the safe establishment of the roots at a depth and later their extension into a large area of soil. This is of fundamental importance in the management of the species and explains the ultimate failure of one crop of seedlings after another in stands which are already moderately crowded or apparently fairly open.

Next in order on such sites we might expect to find Douglas fir, because it, also, produces a deep-rooted seedling. However, we should bear in mind that this species transpires less freely than yellow pine and hence may not be able to tolerate so much insolation. Extended observation shows that it will grow almost anywhere that yellow pine will grow, provided only the seedlings may have shade until they have passed the stage when most susceptible to heat injury. The fact that seedlings start in the shade of and in the densest root area of yellow pine trees shows that this species requires less moisture than yellow pine or, at least, that the moisture is not a controlling factor, and it is apparently for this reason that Douglas fir forms the climax forest except on the warmest yellow pine sites.

Limber pine and bristlecone pine are also, by germination and rooting habit, adapted to well-insolated sites. The sap density of bristlecone pine, however, is apparently considerably higher than that of limber pine, and therefore it succeeds better on cooler sites and on heavier soils. The physiological development of both species and their growth rates are so low that neither can hold a place in the forest in competition with spruce or Douglas fir.

On the other hand we have the poorly insolated sites, commonly described as "cool and moist," which are subject to comparatively slow and wide seasonal changes in their moisture conditions.

Spruce seedlings on account of their growth habit are able early in the season to penetrate the layer of loose organic matter which is in many seasons thoroughly wet only after snow melting. The small seeds germinate at a lower temperature than those of other species. Thereafter the roots show little stimulation to further growth. Even the dryness of the fall period does not appreciably stimulate root growth in the new seedlings, and it is believed that this is clear evidence of the ability of the species to extract water from the soil at a low degree of availability. Possibly because of their generally higher organization, spruce seedlings even prefer a low moisture content which results in greater concentration of the soil nutrients. In such a situation Douglas fir has no theoretical advantage over spruce except in case of a drought so prolonged as completely to dry out the soil layer in which the spruce roots are found. Then the deeper rooting of the fir should, apparently, count in its favor. But in the established forest this can hardly be a material advantage, considering the evenness and depth of the drying where the soil is well occupied by older roots.

The situation with regard to lodgepole is very different from that of our other forest trees, and the writer takes from the evidence the liberty of suggesting either that it is just approaching the physiological status of a full-fledged tree or that it is such a recent migrant to the Rocky Mountain region as to have failed by far to adapt its mode of growth to the poor moisture conditions usually found where its heat requirements are best satisfied. Some of the evidence on the latter point has been presented by the writer (*3*). Clements (*9*) has classed lodgepole

as even more intolerant of shade than yellow pine, and a fairly low photosynthetic efficiency is clearly indicated in this paper. Yet, while evidently demanding a great deal of light as well as moisture during the growing period, it shows no such habit as that possessed by yellow pine, of prompt germination or deep rooting. It is evident, therefore, that it is adapted only to sites with a steady supply of moisture and demands more than can usually be supplied by either of the contrasting situations which have been described. It is probably for this reason that it reproduces readily only where competition is decidedly lacking, adheres to the higher elevations where moisture is more abundant but where its growth rate is surprisingly slow, and has not penetrated to the south where there is a very sharp contrast between the summer rainy period and the clear, dry weather of autumn. In its range there is, to be sure, a generally steady decrease in the precipitation from May or June onward, but this is usually so gradual as to permit a great degree of adjustment.

CONCLUSION

In concluding this paper, it may be said that certain physiological relationships between the species which are of great importance, especially for a proper understanding of forest growth, have been brought out and tentatively established by approach from several angles, but that, from the standpoint of natural reproduction and in relation to all questions of natural distribution of the species, these relative physiological qualities are not shown to be more controlling than some adaptations of form and characteristics of behavior which may be adequately described only by the word "habit." Technical forestry or silviculture might be said to be based on the venerable concept that the several species of the forest vary in their demands for light or their tolerance of shade. This concept is not only not altered by the present results but is confirmed, and the relation of the photosynthetic capacity of a given species to its heat and moisture requirements is made much more clear and definite than it has appeared heretofore. Spruce is shown to be the most efficient of the species considered, not only because of its high photosynthetic capacity, but also because when this capacity is exercised the species automatically becomes economical in its use (by transpiration) of water and at least in this sense has low moisture requirements. At the same time it may be rendered sensitive to excessive temperatures. On the contrary, yellow pine, commonly thought of as very drought resistant, is found to require much light and heat and, with these, to use comparatively large amounts of water per tree of given size. Of course, the facts fit more closely with preconceived ideas when spruce stands are compared with pine stands, the much smaller number of individuals in the pine stand not only compensating for the high individual water use but being a most vital concomitant of this individual requirement. In other words, with the low moisture supply commonly available in the low elevations and warm situations (which alone insure proper development of the pine), wide root spread and an open stand are vitally necessary to insure the water supply of the individual tree. The practical importance of this fact in forest management should be very clear.

It will be seen, then, that these physiological relations principally clarify our conceptions of growth. Spruce is a better grower, a more efficient mechanism for growth, than Douglas fir, and fir more efficient

than pine. In forest planting, where use is made of trees which have been carefully nurtured beyond the stage of greatest susceptibility to heat and drought injury, this difference in the efficiency of our species, particularly the more effective use of water by spruce and fir, may be advantageously employed, the ranges of these species being artificially extended downward, without any injurious effects, while by natural reproduction such extension would be completely prohibited or would be very slow. With even greater care the economic loss resulting from the planting of any species in a situation too high or too cool, or in stands too dense for its proper development, must be avoided.

In short, when it is considered that any cutting of a forest, by admitting more light and creating higher temperatures in the surface soil and more rapid fluctuations of the moisture on which young seedlings are dependent, tends to encourage a species more "hardy" but of a lower order of development than the one which dominates the stand (not necessarily of lower technical value), it is not difficult to see that all forest management hinges on these relative physiological properties for which we have been groping. Finally, it may be said that all of the physiological relations are embodied in the now rather general conclusion of foresters that the highest returns can be had from forestry only when cutting is followed by planting.

This brings us to the original object of the present study, which has been to explain the existing natural forest types, to explain the distribution of the species. As has been pointed out, natural distribution is plainly influenced at present by habits and adaptations which have developed in each species and which to a considerable extent compensate or balance the more deeply embedded physiological qualities. Without again going into the details that have been brought out, we may illustrate to show how these developments affect natural distribution. In figure 5 an attempt is made to show the influence of these developments on distribution, in a broad way. It has been indicated that spruce may be very sensitive to high temperatures, but especially so at a very early age when the seedling is small and tender. In consequence of this weakness, which is a result of its most fundamental organic character, probably for ages no spruce seedling has been able to develop at a low elevation or on a very well-insolated site at a middle elevation. Such sites as are suitable in respect to insolation and heat must show almost invariably quite even moisture conditions and usually soils characterized by a surface layer of litter and humus. Consequently spruce has developed a rooting habit suited to these moisture and soil conditions—a relatively slow and feeble rooting habit which does not suffice for quick establishment under any other conditions. As a further consequence, spruce has evolved very small seeds, there being no need for large, sturdy seedlings or for deep rooting before the seedling may itself manufacture food. It is seen, then, without enumerating any other similar developments, that the species would have great difficulty in extending naturally to any sites other than the cool and moist ones on which it is commonly found. The common conception has been in error only to the extent of assuming that the essential feature of such sites is a large moisture supply. It is now fairly evident that the individual spruce tree does not require a large moisture supply even though this may insure the fullest development of the stand and, in view of this fact, that spruce may be used in planting where the moisture supply is relatively low.

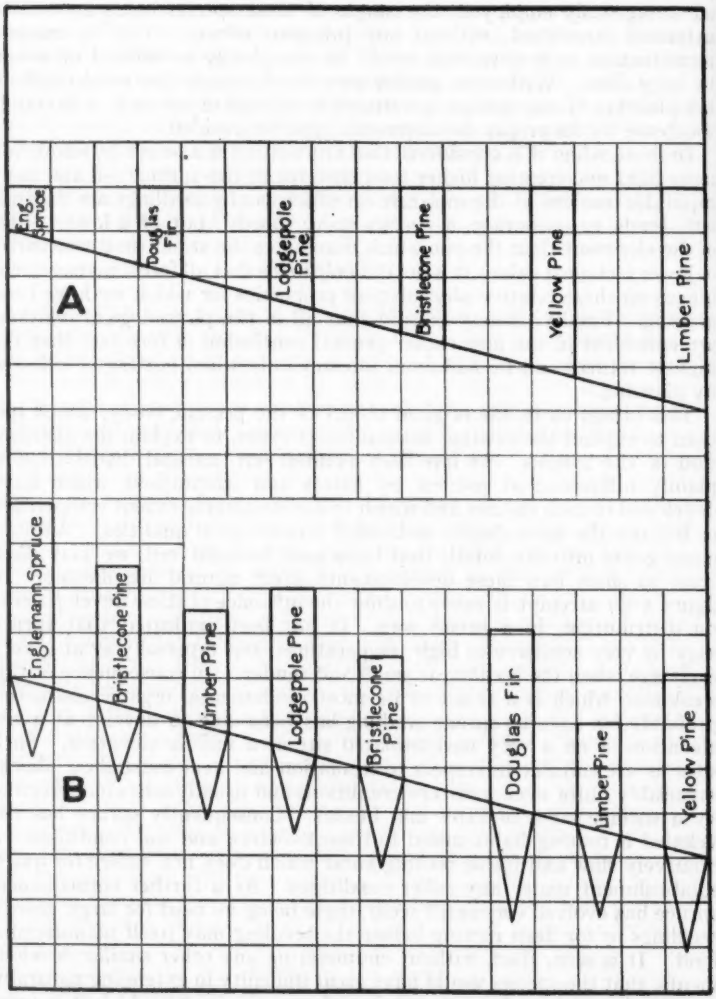

FIG. 5.—A, Theoretical zonation and relative heat requirements on the basis of photosynthetic capacity. B, Actual zonation and relative dominance as influenced by adaptations of roots and foliage.

It is to be hoped that this distinction between the temporary qualities of seedlings which acutely influence natural reproduction and extension of ranges and the more fundamental qualities of the species which later control growth reactions and economic values may be clearly held in mind, since it becomes increasingly apparent as time goes on that the factors controlling reproduction must be considered as almost independent of those controlling later growth.

In a succeeding paper on this subject it is hoped principally to show to what extent the environmental conditions of the different forest types differ and, in the light of what we have so far seen, to weigh carefully the importance of each condition so that those conditions which are really essential to the success of a given species may be clearly understood. The practical application of these facts in forest management may then be shown, it is hoped, in more definite terms.

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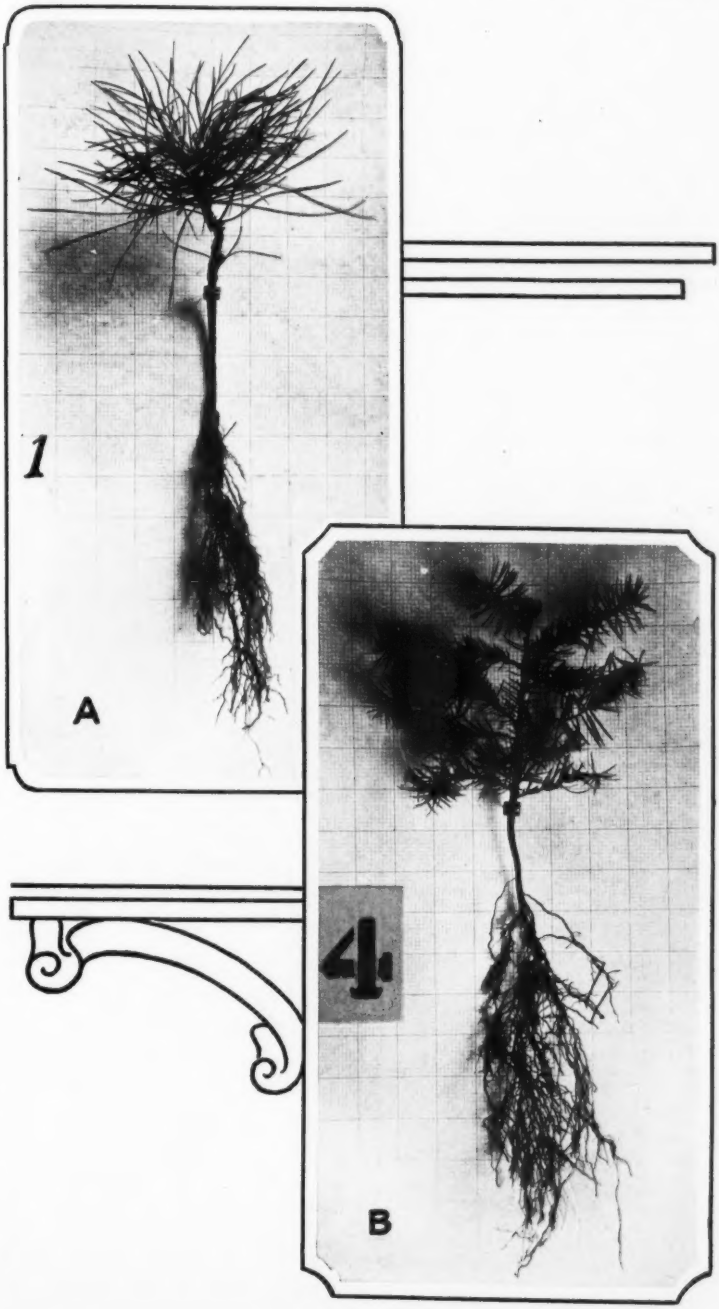
PLATE 1

A.—Tree No. 1, yellow pine, 1917.
B.—Tree No. 4, Douglas fir, 1917.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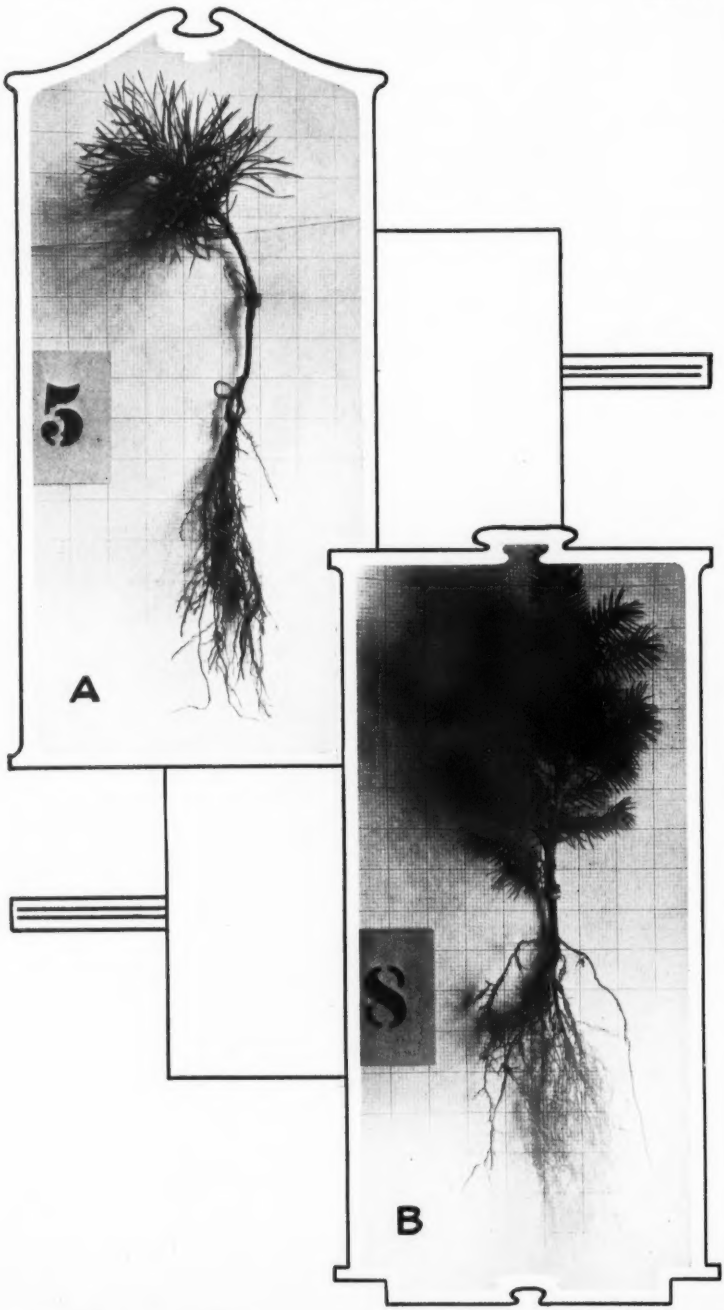
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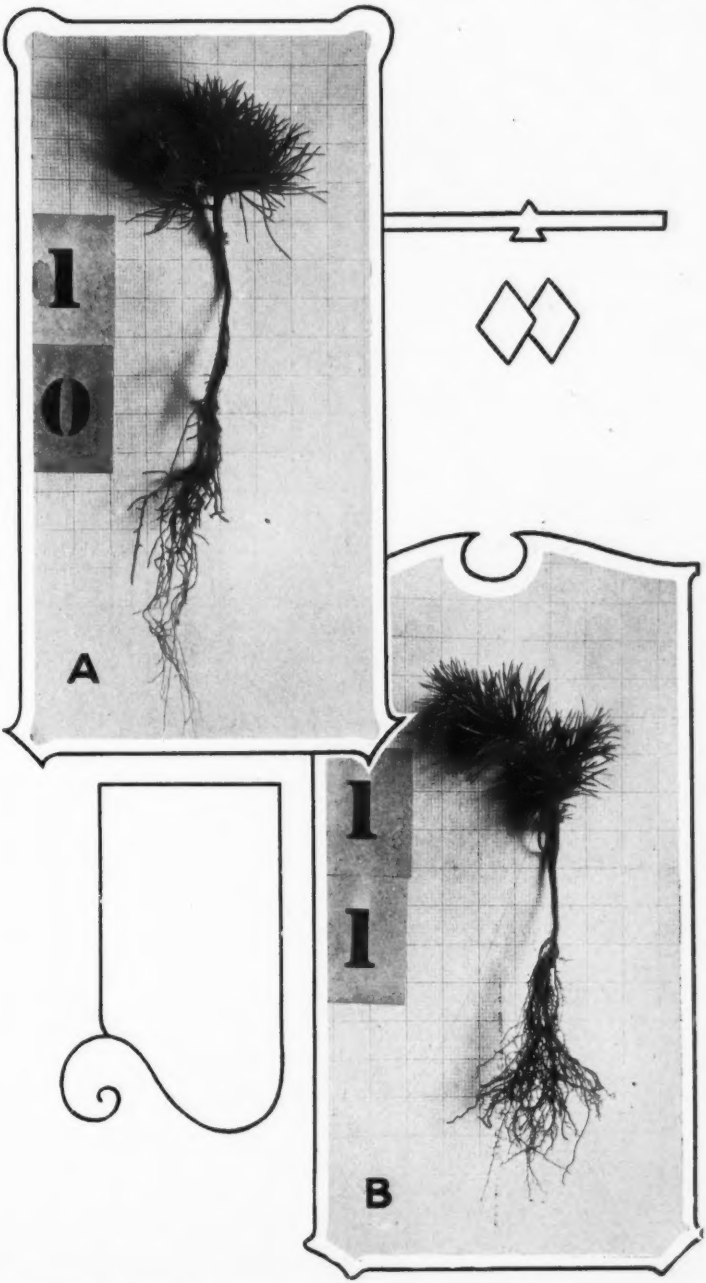
PLATE 2

A.—Tree No. 5, lodgepole pine, 1917.
B.—Tree No. 8, Engelmann spruce, 1917.

PLATE 3

A.—Tree No. 10, limber pine, 1917.
B.—Tree No. 11, bristlecone pine, 1917.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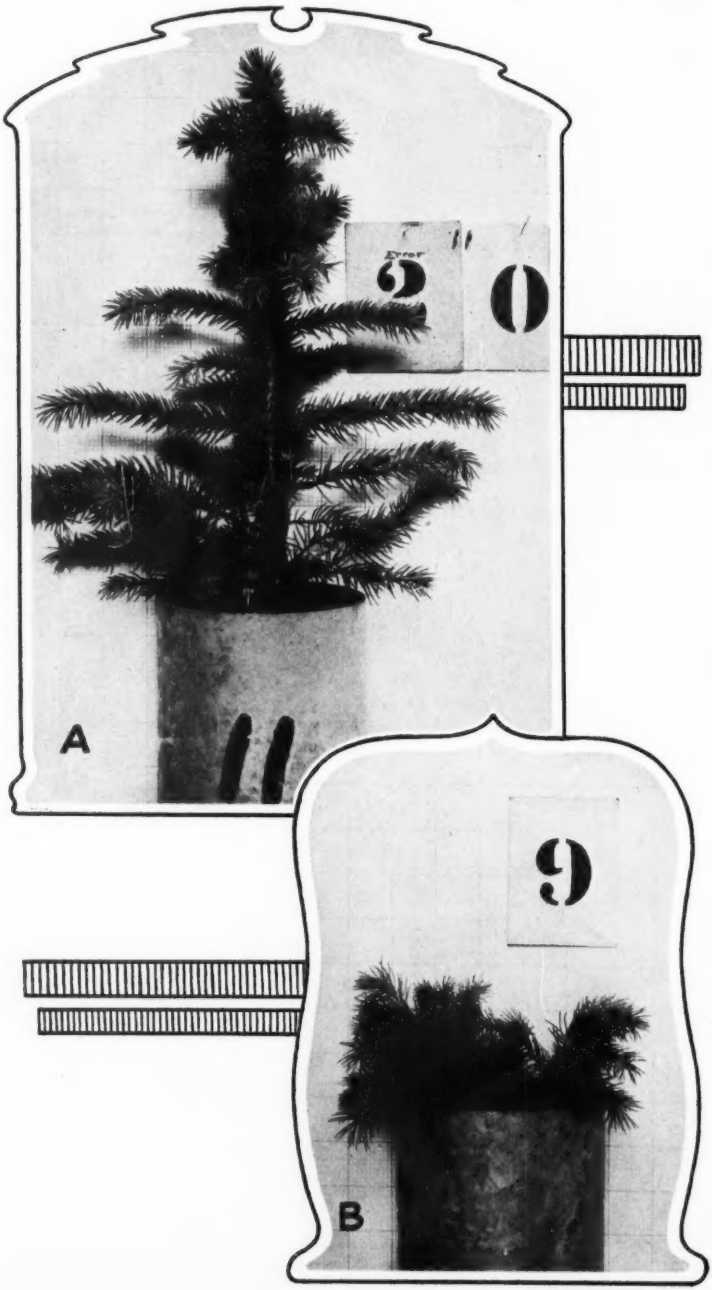
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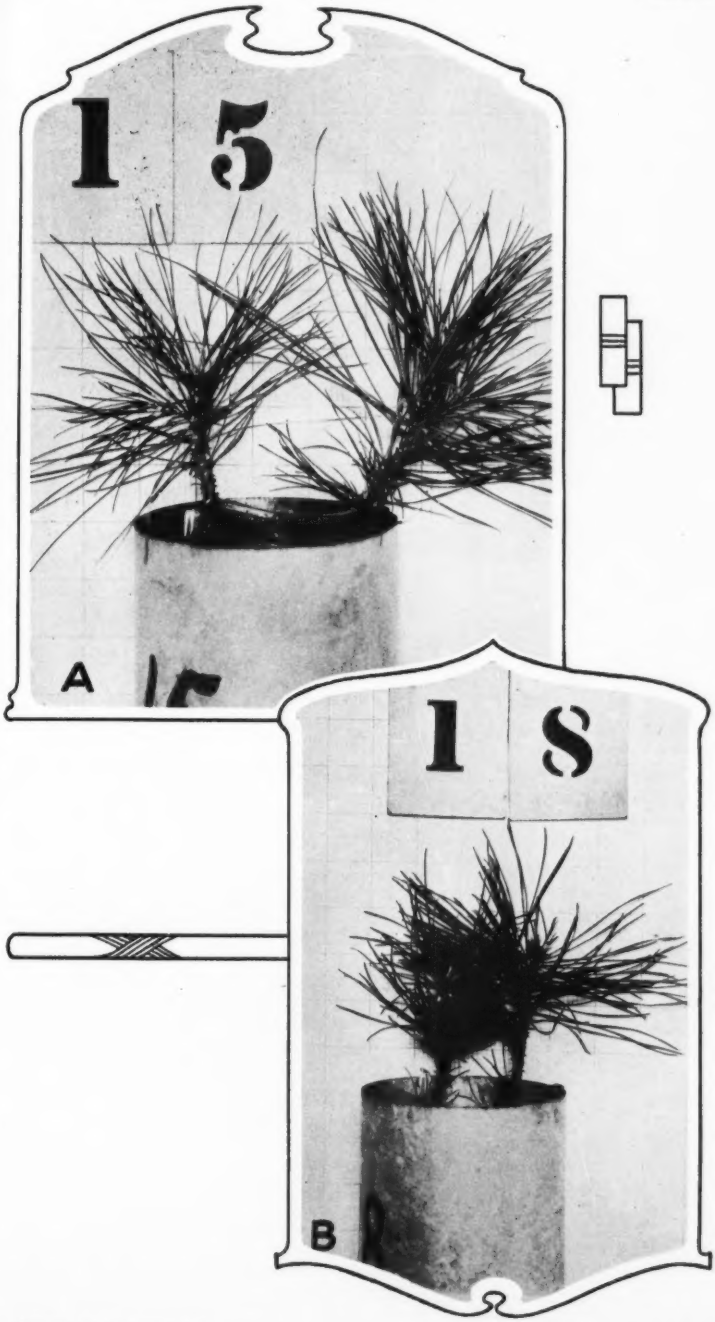
PLATE 4

A.—Large spruce, No. 11, in 1920 transpiration test.
B.—Small spruces in Pot 9, 1920.

PLATE 5

A.—Arizona yellow pine, Pot 15, 192c.
B.—Montana yellow pine, Pot 18, 1920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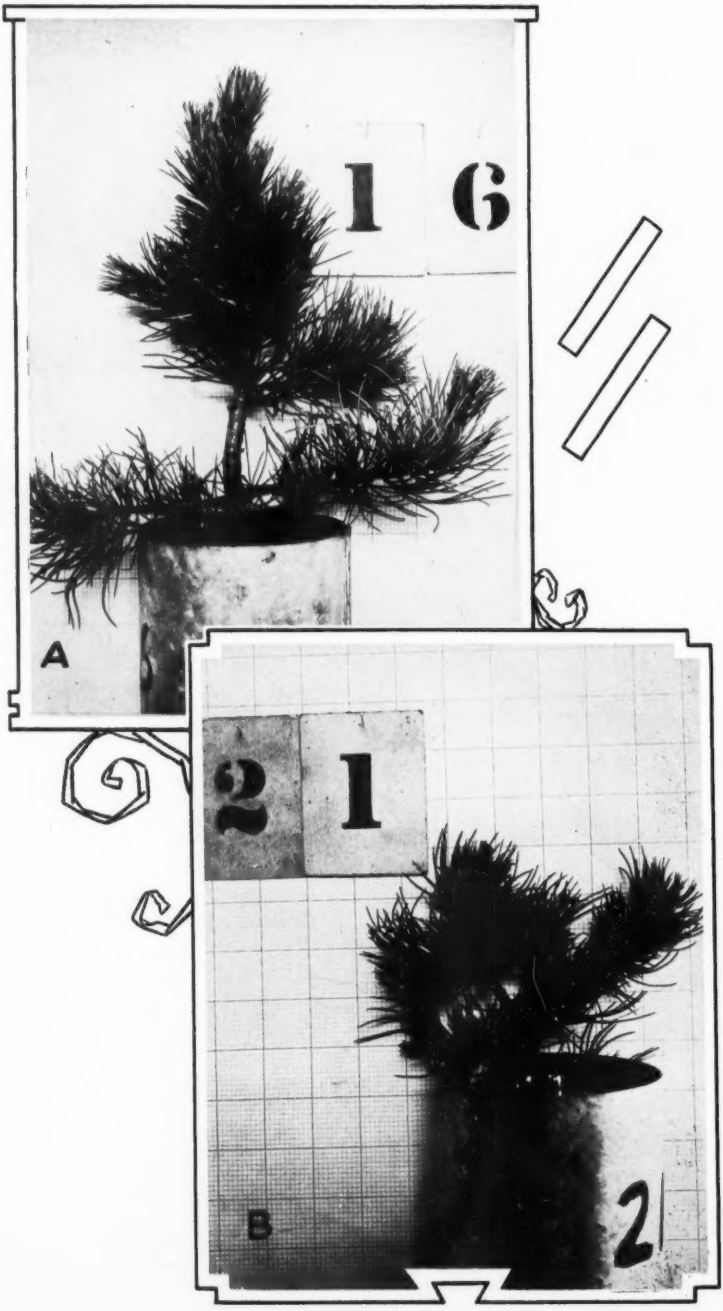
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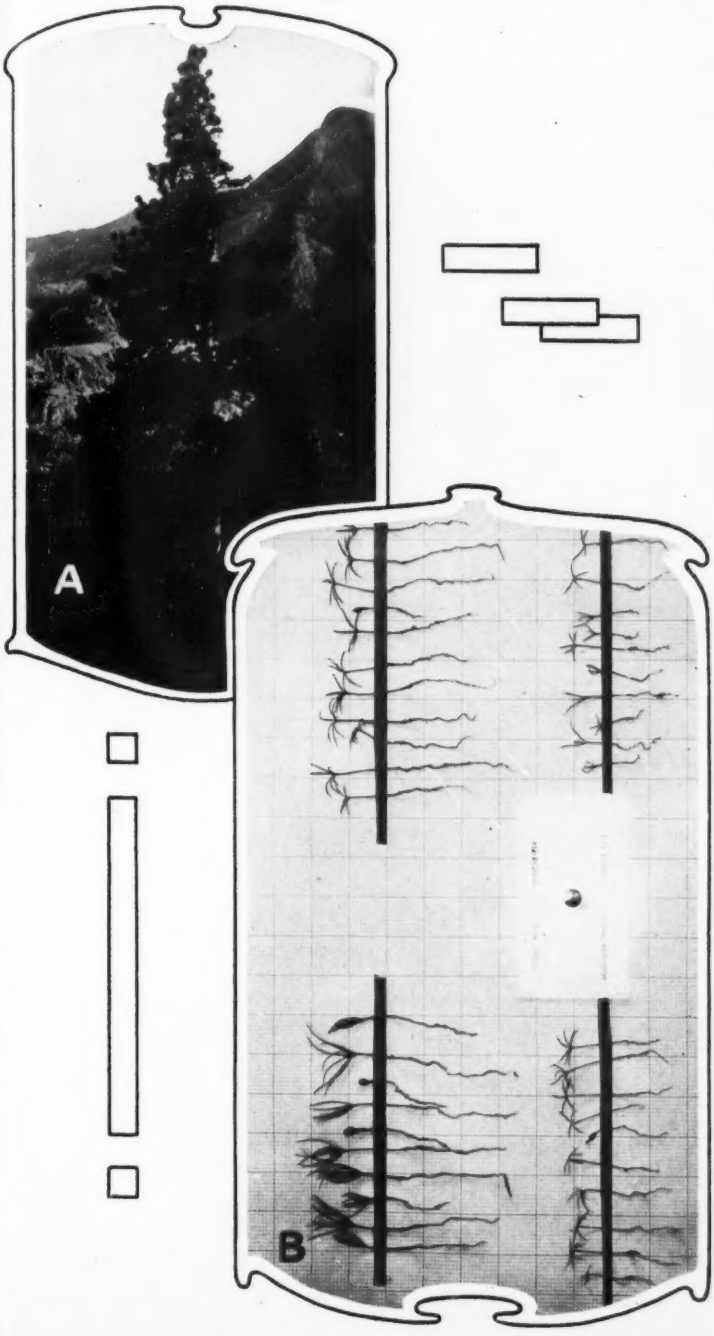
PLATE 6

A.—Vigorous limber pine, Pot 16, 1920. Water requirement 773 units.
B.—Sluggish limber pine, Pot 21, 1920. Water requirement 4,785 units.

PLATE 7

A.—Asymmetrical development of yellow pine, probably resulting from repeated winterkilling of limbs on the west side. (Looking south.) July 2, 1916.
B.—Relative root developments in moist sandy soil of seedlings 30 days after sowing.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A STUDY OF THE INTERNAL BROWNING OF THE YELLOW NEWTOWN APPLE[1]

By A. J. WINKLER

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INTRODUCTION

As a result of the heavy losses of Pajaro Valley apples during the early development of cold storage practices in California, considerable effort by the Bureau of Plant Industry of the United States Department of Agriculture has been directed toward determining the cause of this trouble and toward devising methods of overcoming it. In 1910 Stubenrauch (*16*)[2] indicated a relation between the browning and the temperature of storage, lower temperatures favoring its development. In the report of this bureau for 1920 (*1*) it was stated that there was no relation between the acidity of the fruit and the trouble and that as yet no definite cause could be assigned for this disease.

OBJECTS OF THE INVESTIGATION

The present investigation is an attempt to determine the cause of internal browning, with special reference to (1) field conditions which are responsible for the susceptibility of the fruit and (2) the internal and external factors which are immediately responsible for its development in storage.

DESCRIPTION OF INTERNAL BROWNING

Internal browning as it occurs in the Yellow Newtown apple is a nonparasitic storage disease of the large isodiametric cells of the pulp. In apples stored immediately, regardless of the time of harvest, at −1.1° and 0° C. the browning generally becomes noticeable during the latter part of December, while in apples stored at higher temperatures its first appearance is proportionately later. The writer has not observed its occurrence in apples kept at temperatures of 8.3° or above.

In a cross-sectional view of the apple, the disease is first detectable in more or less elongated areas radiating outward from the central portion of the apple in the region opposite the basal end of the carpels. By cutting the apple in various planes it is apparent that the areas first browned lie adjacent to and radiating outward from the primary vascular bundles.

As the browning becomes more severe, it spreads most rapidly in the region of the secondary vascular bundles. In many specimens it

[1] Accepted for publication July 17, 1922. Submitted as a major thesis to the faculty of the Graduate Division of the University of California, May, 1921, in partial fulfillment of the requirements for the degree of doctor of philosophy.

[2] The writer wishes to express his thanks to Dr. J. C. Whitten, Dr. J. P. Bennett, and Prof. E. L. Overholser for counsel and suggestions during the progress of this investigation. The writer is also indebted to Mr. H. E. Jacob, a graduate student, for assistance in harvesting the fruit, in preparing it for storage, and in making the observations.

[3] Reference is made by number (italics) to "Literature cited." p. 184.

advances far toward the calyx end of the apples in this region before it penetrates more than several millimeters into the pulp laterally near the initial point of browning. Although the browning seems to be confined to the region immediately adjacent to the vascular system in its most rapid penetration of the apple tissue, the bundles themselves are slow to show the browning. The large cells adjacent to the bundles are the first to become discolored. Later the small cells bordering on the bundles are affected, and finally in the advanced stages of the disease the bundles themselves become brown. In its advanced stages the disease may spread to all portions of the pulp, constituting a condition comparable to the usual type of storage breakdown. In the latter condition, however, the browning begins at the calyx end of the apple, involving all of the tissue in its spread, and is accompanied by softening of the affected region.

It often happens that in the very advanced stages the browning spreads into the small thick-walled cells of the epidermis, thus giving the fruit an appearance of being scalded. In its less advanced condition, the disease, however, is detectable only by cutting into the fruit, the skin retaining its natural color and luster and the flesh remaining firm.

METHODS AND PLAN

The storage phases of this problem were carried out in a cold-storage plant consisting of six rooms and two large insulated boxes, making available the following temperatures: 0°, 2.2°, 5°, 8.3°, and 13.9° C. The temperatures were maintained within ±1°, with the exception of the highest temperature, which varied from 12° to 14°. The humidity was practically constant in the different rooms, never varying more than 3 to 4 per cent.

The fruit was obtained from the Rogers Bros.[4] orchards, which are located about 1 mile east of Watsonville, Calif. The apples were packed and labeled under the respective trees from which they were picked. The fruit was not sorted, but represented tree-run apples, with the exception of the third picking in 1919, which was made up of grade "B" apples. The boxed apples were then expressed to Berkeley.

Three pickings were made each season. The earliest seasonal picking, 15 lots, was made just as the regular picking season was beginning. The second seasonal picking, a similar number of lots, came at about the middle of the normal picking season. The third picking was delayed until the close of the harvesting season. In each case a "lot" represented the fruit from a single tree.

The first two pickings for both seasons were stored the third or fourth day after picking. The storage of the last picking, however, was delayed approximately three weeks each season due to the slow shipment.

After arriving at the storage plant, the lots were divided into the required number of sublots for the several individual experiments. The sublots of approximately 80 specimens each, unless stated otherwise in connection with the separate tests, were stored in apple boxes. Sufficient space was always maintained between the boxes for normal ventilaton. Not only were apples of the same lot used in each experiment and in the control, but the apples of each test were placed under as nearly identical conditions as possible.

[4] The writer is greatly obliged to Messrs. C. J. Rogers and Marion Rogers for their interest and hearty cooperation in the work

As internal browning affects the flesh of the fruit, it was necessary to cut the apples in order to make observations upon their condition. The apples were cut perpendicularly to the axis of the core in a plane which passed approximately through the junction of the carpels with the stem. By cutting in this plane the browning was always detected, if present.

Observations upon the browning were made at monthly intervals beginning with February 2 for the 1919–20 season and with January 8 for the season of 1920–21. Twenty specimens from every sublot were cut at each of the four seasonal cuttings. By cutting several hundred apples on numerous occasions it was found that the error of observation in cutting only 20 specimens ranged from 2 to 5 per cent. When the results for the four seasonal cuttings were averaged, this error was reduced to 1 to 2 per cent. In recording the observations the following terms were used to designate the degree of browning:

1. NORMAL.—No browning apparent to the unaided eye; less than 0.1 per cent of cells affected. (Pl. 1, A.)

2. TRACE BROWNING.—Browning recognizable in the torus but not of sufficient intensity to lessen the market quality; 0.1 to 0.6 per cent of cells affected. (Pl. 1, B.)

3. SLIGHT BROWNING.—Browning in a sufficient degree of intensity to lessen the market quality of the fruit but not to such a degree as to make the apples objectionable for culinary purposes; 0.6 to 10 per cent of cells affected. (Pl. 1, C.)

4. MODERATE BROWNING.—Browning of such an intensity as to render the apples unsuitable for ordinary culinary purposes. At this stage of browning the tissue was more generally discolored throughout the torus, from 10 to 30 per cent of the cells being brown. (Pl. 1, D.)

5. SEVERE BROWNING.—This term refers to a degree of browning which upon cutting gave the apples an appearance of being rotten within. In these apples the structure of the tissue exhibited a marked degree of disintegration in all portions of the specimen; 30 per cent or more of the cells affected. (Pl. 1, E.)

PRESENTATION OF DATA

RELATIVE DEGREE OF INTERNAL BROWNING EXHIBITED BY YELLOW NEWTOWN APPLES GROWN IN THE PAJARO VALLEY AND ELSEWHERE

During this study apples were also obtained from other localities in California and from important districts in other States where this variety is successfully grown to determine whether or not the browning was confined solely to apples grown in this valley. All of the fruit was shipped to Berkeley by express and was then stored at 0° C. under the same conditions as the fruit from the Pajaro Valley.

The figures obtained in these tests indicate that Yellow Newtown apples generally are more or less susceptible to internal browning. The disease in apples from points other than the Pajaro Valley, however, has not been sufficiently severe to render it an economic problem.

The fact that all the apples showed browning would seem to indicate that either something peculiar to the variety makes it susceptible to browning or that the trouble lies in the regions in which it is at present most extensively grown. Both of these conditions appear to be more or less responsible for the browning. The fact that other varieties of apples grown in the same districts, with the exception of the Pajaro Valley, are immune to this disease would at least suggest that the Yellow New-

town exhibits a varietal characteristic of susceptibility to internal browning.

The effect of the region in which the fruit is grown upon its susceptibility to browning is indicated by the fact that several varieties of apples such as the Yellow Bellflower and the Red Pearmain, which normally show no tendency to brown, are susceptible to this disease when grown in the Pajaro Valley. These varieties do not brown when grown in any of the other Yellow Newtown districts. Furthermore, the Yellow Newtown, when grown in this valley is much more susceptible to internal browning than when grown elsewhere. The climatic conditions of the Pajaro Valley, therefore, seem to exert an influence upon the development of apples which has not been shown to occur elsewhere and which renders them susceptible to this disease.

RELATION OF TIME OF HARVESTING TO INTERNAL BROWNING

The importance of the time of harvesting of the fruit in the control of nonparasitic diseases of the apples has been stressed by Powell and Fulton (*13*), Brooks, Cooley, and Fisher (*4, 5, 6*), and others. Correspondence with cold-storage managers showed that some of them believe that internal browning is, at least in part, the result of picking the apples too green. The riper apples which have a higher sugar content, according to these men, are more resistant to browning.

An investigation of the effect of the time of harvesting upon internal browning was started in the season of 1919–20. Three pickings of fruit were made for both this and the 1920–21 seasons. The fruit of the first picking was "hard green" in maturity and of a solid green color; that of the second picking was "firm green" and signs of the yellow color were becoming evident; while the fruit of the last picking was "overripe" for harvesting and showed a considerable amount of yellow over the entire surface. The fruits for each lot were picked from all portions of the same trees at each of the pickings and were then stored under identical conditions at 0° and 2.2° C. The effect of the time of harvesting upon the severity and rate of browning are shown in Tables I and II.

The figures of Table I indicate that the later-picked fruit browned much more severely in every test. In the case of the apples stored at 2.2° C. during the season 1919–20, the actual figures in the table show little difference in the amount of browning. When the difference in the storage dates is taken into account, however, a considerable difference in favor of the earlier pickings becomes apparent. In the other cases the relation of time of harvest to the browning is obvious.

The figures of Table II indicate that the fruit of the last picking browned two and one-half times as rapidly as that of the second picking and the fruit of the second picking browned one and one-half times as rapidly as that picked at the beginning of the harvest season.

The sugar content of the fruit picked at the time of harvest September 26, October 16, and November 6 was 9.4, 10, and 11.4 per cent, respectively, which appears to indicate that a higher sugar content favors browning. By analyzing a large number of samples, however, it was shown that the sugar content does not influence the resistance or susceptibility of the fruit to browning. It might also be expected that the change in acidity of the fruit of the later picking, due to its more mature condition, and the subsequent prolonged storage would affect its resistance to browning. This was found not to be true. Although the titrable

acidity decreased with maturity and subsequent storage, the active acidity as indicated by the P_H value of the expressed juice remained practically constant. This possibly accounts for the fact that the decrease in total acidity does not influence the resistance of the fruit to browning.

TABLE I.—*Relation of time of harvesting of the fruit to internal browning*

Storage temperature.	Date of picking.	Date of storage.	Condition of fruit at end of storage period, approximately Apr. 1.				
			Normal.	Trace.	Slight.	Moderate.	Severe.
SEASON OF 1919–20							
°C.			*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
2.2	Sept. 28	Oct. 2	45	50	5	0	0
2.2	Oct. 18	22	50	45	5	0	0
2.2	Nov. 22	Dec. 8	45	45	10	0	0
0	Sept. 28	Oct. 2	40	20	15	15	10
0	Oct. 18	22	20	45	20	10	5
0	Nov. 22	Dec. 8	15	35	20	10	20
SEASON OF 1920–21							
2.2	Sept. 27	Sept. 30	75	10	5	10	0
2.2	Oct. 16	Oct. 19	60	35	5	0	0
2.2	Nov. 6	Nov. 26	5	45	30	20	0
0	Sept. 27	Sept. 30	30	40	20	10	0
0	Oct. 16	Oct. 19	20	45	25	10	0
0	Nov. 6	Nov. 26	0	30	45	25	0

TABLE II.—*The relation of time of harvesting of the fruit to the rate of development of browning*

Storage temperature.	Date of picking.	Condition of fruit.	Weeks in storage.	Marketable.[1]	Unmarketable.
°C.				*Per cent.*	*Per cent.*
0	Sept. 26	Hard green	20	60	40
0	Oct. 16	Firm green	15	60	40
0	Nov. 6	Overripe	6	60	40

[1] Marketable fruit includes both normal and trace browned specimens.

EFFECT OF TEMPERATURE UPON INTERNAL BROWNING

STORAGE TEMPERATURE

At the time apple storage was introduced in California, investigations by Powell and Fulton (*13*) had brought the cold-storage men to the general belief that all apples could be stored most successfully at −5/9° to 0° C. It was soon found, however, that great losses were incurred through the deterioration of Yellow Newtown apples of the Pajaro Valley at these temperatures. As a result of these losses investigations (*16*) were undertaken which brought about the storage of all the Pajaro Valley apples at 2.2°.

At the University of California, where apples of the Pajaro Valley have been stored at −1.1°, 0°, and 2.2° C. since 1916, it has been found that considerable browning occurs after the first of February, even in the fruit stored at 2.2°. Aside from the browning, however, these apples keep satisfactorily. It was, therefore, thought advisable to store apples at various temperatures above 2.2° in order to determine (1) the lowest temperature at which internal browning will not develop during the normal storage period and (2) whether or not the temperature which is sufficiently high to prevent internal browning is also sufficiently low for practical storage purposes.

During the season of 1919–20 the only other temperature available above 2.2° C. was that of room temperature. However, in 1920–21 apples were stored at 5°, 8.3°, 13.9°, and 21° in addition to the usual temperatures used for these apples. In the 1919–20 season 15 lots of the second picking were used in these tests, while in 1920–21 two lots of fruit from the same two trees for each of the first two pickings were used. Since all the apples browned in relatively the same proportions at each of the temperatures, only the averages for the second picking are given in Table III.

TABLE III.—*Effect of storage temperature upon internal browning*

Storage temperature.	Condition of fruit after 4 months' storage.				
	Normal.	Trace.	Slight.	Moderate.	Severe.
°C.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
SEASON 1919–20					
−1.1[a]	15	60	20	5	0
0	15	35	30	15	5
2.2	40	55	5	0	0
21	100	0	0	0	0
SEASON OF 1920–21					
−1.1	15	35	25	20	5
2.2	40	35	20	5	0
5	95	5	0	0	0
8.3	100	0	0	0	0
13.9	100	0	0	0	0
21	100	0	0	0	0

[a] There was considerable freezing at −1.1°C. in the early part of the storage season, which retarded the browning.

The figures of Table III show a definite relation between the amount of browning and the temperature of storage. The effect on browning of only a few degrees change in temperature is very striking. At 0° C., for instance, in the 1920–21 season, only 15 per cent of the fruit remained normal, while at 2.2° 40 per cent was normal, and at 5° 95 per cent of the fruit was normal. Browning did not occur in any of the fruit stored at a temperature of 8.3° or above.

It becomes manifest, therefore, that internal browning does not occur at a temperature a few degrees above that used in the commercial storage

of apples. Furthermore, the Yellow Newtown is known to be one of the best keeping apples, and it may be held quite satisfactorily in basement storage until May if well-matured, sound fruit is used. Thus it appears that where prompt storage under uniform conditions is possible the fruit can safely be held at temperatures sufficiently high to prevent browning without other forms of deterioration developing. In commercial practice, however, it would probably not be expedient to store apples above 5° C. It would, nevertheless, be advisable to store these apples at or just below this temperature.

The browning was not only increased in severity as the temperature decreased below 5° C. (as shown by the figures in Table III) but its development was also more rapid. This relation of temperature of storage, with the time of initial appearance and the subsequent development of the browning, is illustrated by the graphs in figure 1.

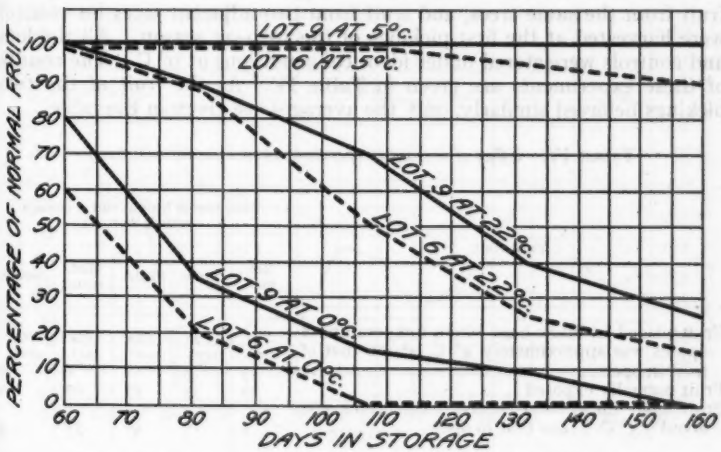

FIG. 1.—Effect of temperature upon the rate of development of internal browning.

ORCHARD TEMPERATURE

In the early investigations upon internal browning, letters, in the form of questionnaires, were sent to the leading fruit men of the Pajaro Valley. In reply to the question asking when the browning was most prevalent, many of the fruit growers attributed its occurrence to the cold, foggy weather which characterizes this valley during the latter part of July and the early part of August, just at the time the fruit is growing most rapidly.

With this observation as a basis, experiments were started in the spring of 1920 to determine the effect of orchard temperature and fog upon internal browning. During the first week of May, 1920, a tent of black cambric cloth was erected over a single average tree which bore a normal set of fruit. The sides of the tent came within about 8 feet of the ground. Thus, all the branches with fruit were shaded continuously. At the same time the tent was erected, 100 individual apples on an adjacent tree were placed in black cloth bags. A similar number of apples on adjoining trees were placed in black bags on the first of June and July, respectively.

Owing to the lack of necessary equipment, it was impossible to measure the exact effect of the tent and black bags upon the light intensity. Nevertheless, it is thought that the light exclusion as such is negligible, since it had little or no effect upon the amount of browning as shown by the figures of Table V. The number of foggy days might, of course, exert an indirect effect by influencing the temperature.

The effect of the tent and black bag upon the temperature, however, was very striking. The mean daily temperature at the core of the bagged fruit, as indicated by self-recording thermometers, was from 2.5° to 5.5° C. higher than that of apples normally exposed. In the case of the tented tree, a lower mean temperature was maintained by the shading and lack of free circulation of the air. Here the temperature, as recorded by accurately regulated thermograph instruments, was found to be from 2° to 4.5° lower than that for a similar position in an adjacent untented tree.

The fruit from the tented tree, the bagged and the normally exposed fruit from the same trees, and fruit from two adjacent trees for control were harvested at the first pickings of the 1920–21 season. All the lots and controls were stored under identical conditions at 0° C. The results of these experiments are given in Table IV. As the fruit of the two pickings behaved similarly, only the averages are given in the table.

TABLE IV.—*Effect of orchard temperature upon internal browning*

Treatment.	Condition of fruit at end of storage period, Apr. 1.				
	Normal.	Trace.	Slight.	Moderate.	Severe.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
Fruit placed in black bags where temperature of apples was approximately 4° C. above that of fruit in open	95	5	0	0	0
Fruit normally exposed	30	35	25	10	0
Fruit from under tent where temperature was about 3.5° C. below that in open	5	15	40	35	5

The figures of Table IV show a very definite relationship between the orchard temperature and internal browning. A daily mean temperature of 4° C. above the normal temperature of the orchard practically prevented the browning, while a mean temperature of 3.5° below the normal mean orchard temperature greatly increased the amount of browning over that which occurred in the normally grown fruit.

This relation between orchard temperature and the amount of browning becomes more impressive when the temperature records of this valley are compared, for the years of severe and of moderate or no browning for this region, with the temperature records of other districts where this variety of apple grows satisfactorily and where the browning is not a problem. The graphs in figure 2 represent the mean temperature for June, July, August, and September for the Pajaro Valley, Calif.; Albemarle County, Va.; and Rogue River Valley, Oreg. If the record for the Pajaro Valley is considered, it will be seen that in 1908 and 1914, years in which very heavy losses through internal browning occurred, the mean temperature for these four months was very low. For 1909, 1910, 1915, and 1916, when the mean temperature for these

growing months was nearly normal, the severity of the browning was less. In 1912, 1913, 1917, and 1918, years of higher mean temperature for the months of rapid growth, there was no browning in the commercial storage plants. Comparing the temperature records of the Pajaro Valley with those of Rogue River Valley, Oreg., and Albemarle County, Va., it is found that these regions have a mean temperature of 2.8° and 6.2° C. higher, respectively, than that of the Pajaro Valley.

Results which further confirm this relation of orchard temperature to the browning were obtained by collecting fruit from well-exposed and from shaded portions of the tree. Two lots of fruit were collected from the upper southwest periphery of two trees where the fruit received the maximum effect of the sun's rays, while two other lots were picked from the lower north, shaded portion of the same trees where the fruit was continuously in the shade. These lots were stored side by side at 0° C. The results of this experiment are given in Table V.

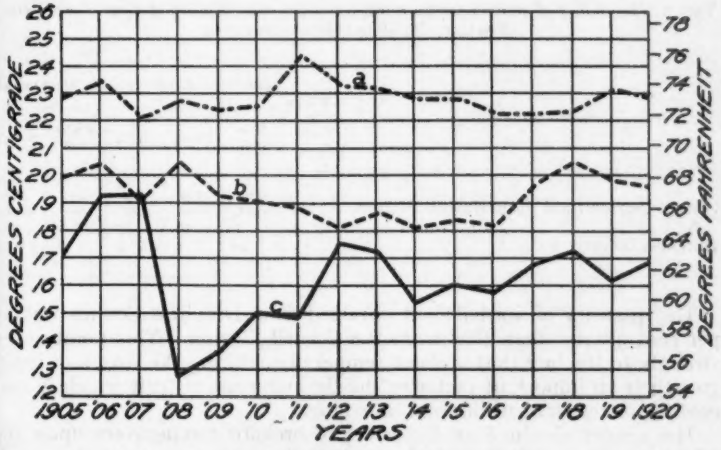

FIG. 2.—Mean daily temperature for June, July, August, and September from 1905 to 1920 for (*a*) Albemarle County, Va., (*b*) Rogue River Valley, Oreg., and (*c*) Pajaro Valley, Calif. (From climatological data reports, U. S. Dept. of Agr. Weather Bureau 1905 to 1920.)

TABLE V.—*Effect of exposure of the fruit on the tree upon internal browning*

Location of fruit on tree.	Condition of fruit at end of storage period.				
	Normal.	Trace.	Slight.	Moderate.	Severe.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
Well exposed, on upper southwest periphery of the tree	35	55	10	0	0
Shaded, on lower north side of the tree	15	50	25	10	0

These figures show that the quantity of normal fruit from the well-exposed portions was 20 per cent greater than that obtained from the shaded portions of the same trees. While no records were taken with regard to the temperature of the fruit at the different exposures, it is safe

to assume that the mean daily temperature of the well-exposed fruit was somewhat higher than that from the shaded side of the tree.

Further evidence which seems to show the definite relation of low orchard temperature to the development of internal browning in this variety of apple is brought out in the results obtained from the fruit received from New York State. One lot of fruit was obtained from the Cornell Agricultural Experiment Station, where the mean summer temperature is only slightly more than 1° C. above that of the Pajaro Valley and where cloudy or rainy weather frequently prevails during the growing season. The other lot was obtained from northern part of Dutchess County, N. Y., where the mean summer temperature is approximately 3.2° higher than that of the Pajaro Valley and where, as a rule, there is an absence of cloudy or rainy weather during the growing season. After express shipment to Berkeley, Calif., these lots were stored under identical conditions at 0°. The figures in Table VI give the results of this test.

TABLE VI.—*Effect of orchard temperatures upon the susceptibility of New York Yellow Newtown apples to internal browning*

Source of the fruit.	Weeks in storage.	Condition of fruit at end of storage period.				
		Normal.	Trace.	Slight.	Moderate.	Severe.
		Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
Cornell Agricultural Experiment Station, N. Y.	14	5	50	15	0	0
Dutchess County, N. Y.	13	100	0	0	0	0

The quantity of normal fruit obtained from Dutchess County was 95 per cent greater than that from the Cornell Station. This points very strongly to the fact that orchard temperature during the season of rapid growth is an important factor in the development of fruit which is susceptible or resistant to internal browning.

The results obtained on the effect of orchard temperature upon the subsequent susceptibility of the fruit to browning point to the possibility that the mean temperature for the growing season in the Pajaro Valley hovers around the lower limit for the normal development of this variety of apple.

RELATION OF ESSENTIAL OILS TO INTERNAL BROWNING

Before taking up a discussion of the data with regard to the relation of essential oils to internal browning, a few references will be made to previous work on the effect of essential oils and allied substances upon the cell.

Dixon and Atkins (*8*) have shown that anaesthetics increase the permeability of the plasma membrane, for the cell sap is readily expressed after their application. When applied for this purpose, however, the anaesthetics were toxic and their effect irreversible. Since a distinctive mark of an anaesthetic is the reversibility of its action, Osterhout (*12*) made measurements upon tissues to determine whether the increase in permeability, usually observed to follow their application, is due to the anaesthetics or to toxins. He concludes that the anaesthetics produce

a decrease in the permeability which is reversible and the subsequent increase in permeability is due to the accumulation of toxic substances as a result of the action of the anaesthetics. In 1910 Armstrong (*2*) and his co-workers showed that, under the influence of anaesthetics and certain other substances which they called hormones, reactions occur in the cells which indicate that the enzyms and their substrates were brought into contact. Among the results of this mixing of the enzyms and substrates, as observed by these workers, was an oxidation which resulted in pigmentation. These workers also state that these phenomena are constantly taking place in the plant but that under normal conditions their products are passed off before they become injurious. Under abnormal conditions, however, they may accumulate in sufficient amount to greatly hinder the activities of the tissues and eventually to cause the death of the cells. Giglioli (*9*) found that essential oils markedly influence the movements of water, enzyms, and soluble substance through the cell membrane. Later, Giglioli (*10*) also demonstrated that the enzyms could be removed from yeast cells by rendering them permeable with essential oils.

During the past year Power and Chestnut (*14*) have isolated the essential oils of the apple. They have shown conclusively that essential oils are being produced continuously by the apple in sufficient quantities to be detected. In 1919 Brooks, Cooley, and Fisher (*5, 6, 7*) found that apple-scald, a nonparasitic storage disease which is generally confined to the surface of the fruit, was apparently due to volatile substances which are produced by the fruit when held for some time under the more or less abnormal conditions of storage. In substantiating this contention, they present data which show that the disease is reduced to a minimum by removing these volatile substances from the fruit by air circulation or by storing the fruit in wax or oil wrappers that are known to be good absorbents of essential oils.

After making observations upon the appearance of the fruit in internal browning and in advanced stages of apple-scald, the writer became convinced from the firmness of the tissue and the way in which these diseases spread into the flesh of the fruit that there is a similarity between these two storage diseases. Histological examinations of affected tissues further emphasized the analogy which exists between these diseases. For the histological studies, sections of the apple torus were cut by means of a freezing microtome. The sections were dropped directly from the razor into acidified absolute alcohol which fixed them and prevented any additional browning. They were then passed through xylol and mounted in Canada balsam without staining. The examination of a large number of cells brought out a very striking similarity between browning and scald in the progress of the discoloration in the tissues as well as in the individual cell. In the tissue there was no regularity in the spread of the disease from one cell to the other, since isolated cells showing browning were always found to be scattered among the normal cells near the regions of scald or browning. In the cells in which the progress of the browning could be followed it was found to be identical in the two diseases. The browning started at the periphery of the cell and from there spread to all parts of the cell along the more concentrated strands or areas of cytoplasm. The discoloration was, as a rule, more intense in the region of the nucleus which is near the surface in the apple cells. Plasmolysis accompanies the advanced stages in browning, until, in the very severe stages, the

protoplast occupies only a small fraction of the cell. The cell wall remains unchanged. As a result of this apparent similarity between these diseases the writer carried out experiments upon the control of internal browning which had proved effective in reducing the amount of apple-scald.

AIR MOVEMENT AS A PREVENTIVE OF INTERNAL BROWNING

Two sublots of apples of the 1919 and 1920 seasons were stored in slat boxes, one box being wrapped in the ordinary manner while the other was stored without wrapping. These apples were then ventilated by a fan for 10 to 20 minutes twice each week. In the season of 1920–21, apples were also placed in sealed containers which were fitted with tubes for pulling air through with a filter pump. Thirty-three apples of the same lot were placed in each container, while a similar number in ordinary storage served as a control. The results of these experiments are given in Table VII.

TABLE VII.—*Effect of air movement upon the development of internal browning*

Treatment.	Season.	Storage temperatures.	Condition of fruit at end of storage period.				
			Normal.	Trace.	Slight.	Moderate.	Severe.
		°C.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
Apples in common storage	1919–20	2.2	35	60	5	0	0
	1920–21	0	5	75	15	5	0
Apples in slat box, wrapped, ventilated 10 to 20 minutes twice each week	1919–20	2.2	45	50	5	0	0
	1920–21	0	60	40	0	0	0
Apples in slat box, not wrapped, ventilated 10 to 20 minutes twice each week	1919–20	2.2	100	0	0	0	0
	1920–21	0	75	25	0	0	0
Apples wrapped but not ventilated with filter pump [1]	1920–21	0	0	5	10	50	35
Apples wrapped, air drawn through slowly with filter pump [1]	1920–21	0	70	25	5	0	0
Apples not wrapped, air drawn through slowly with filter pump [1]	1920–21	0	70	30	0	0	0

[1] Apples sealed in cans with arrangement for slow renewal of air.

The figures of Table VII indicate that where ventilation was employed there was a great decrease in the amount of fruit that exhibited the disease. The figures also show a very definite relation between the effectiveness of the ventilation and the severity of browning, for in every case the wrapped fruit showed more browning than did that which was not wrapped. This definite reduction by ventilation in the amount of browning would seem to indicate that the trouble is favored by the accumulation of deleterious substances which were removed by both the intermittent and the slow but continuous air movement.

GAS ABSORBENTS AS AGENCIES IN THE PREVENTION OF INTERNAL BROWNING

Several sublots of the 1919 and 1920 crops of apples, picked October 18, were stored at 0°C. in oil wrappers which were known to be good absorbents of gases. For this purpose commercial 10 by 10 inch apple wrappers were saturated with the given wax or oil. The number of treatments included and the results of these tests are given in Table VIII.

TABLE VIII.—*Effect of gas absorbents upon the development of internal browning of apples stored at ° C.*

Treatment.	Condition of fruit at end of storage period.									
	Season of 1919-20.					Season of 1920-21.				
	Normal.	Trace.	Slight.	Moderate.	Severe.	Normal.	Trace.	Slight.	Moderate.	Severe.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
Cocoa-butter wrapper	30	45	20	5	0	50	40	10	0	0
Olive-oil wrapper	40	60	0	0	0	55	45	0	0	0
Wesson-oil wrapper	50	50	0	0	0					
Mazola-oil wrapper	50	45	5	0	0					
Vaseline wrapper	70	25	5	0	0	45	55	0	0	0
Lard-tallow wrapper						15	75	10	0	0
Paraffin wrapper						5	80	15	0	0
Control, common wrapper						5	85	10	0	0
Control, no wrapper						5	65	20	10	0
Average for oil wrappers	48	46	6	0	0	34	57	7	0	0
Average for the controls	20	25	25	20	10	5	75	15	5	0

These data indicate that the amount of browning can be reduced by employing agents which absorb essential oils or emanating gases. Since all the tests as well as the controls were stored in identical boxes and under as nearly as possible the same conditions in the storage rooms, the beneficial effect of the oil wrappers must lie in their ability to prevent the accumulation of injurious substances. There was a reduction of about 30 per cent in the number of specimens showing browning in each case.

At the time the fruit picked October 18, 1920, was stored, 10 portions of 33 specimens each of lot "A" were placed in sealed containers with various wrappers as listed in the table. This was thought to be a more accurate method of determining the effectiveness with which certain absorbents control the disease. It would seem logical to assume that there was always a considerable supply of esters or other deleterious material in the storage room; hence the wrappers in the open boxes should absorb these as readily, if not more so, than the substances from the individual specimens which were wrapped, thereby dissipating their ability to function as active absorbents. In the sealed containers, however, the esters to be absorbed were more nearly confined to those produced by the inclosed fruit, and this should materially lengthen the period during which the oils in the wrappers would act as absorbing agents. The results of these tests are given in Table IX.

TABLE IX.—*Effect of gas absorbents upon the development of internal browning*

Treatment.[1]	Condition of fruit after 11 weeks at 0° C.				
	Normal.	Trace.	Slight.	Moderate.	Severe.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
Ordinary commercial wrappers	0	5	0	60	35
Wrappers impregnated with cocoa butter	65	35	0	0	0
Wrappers dusted with animal charcoal	45	50	5	0	0
Wrappers dusted with silica powder	0	5	5	45	45
Wrappers impregnated with olive oil	55	45	0	0	0
Wrappers impregnated with paraffin	25	65	10	0	0
Wrappers impregnated with lard-tallow mixture	30	60	10	0	0
Wrappers impregnated with vaseline	65	35	0	0	0
Commercial wrappers, with 225 cc. concentrated potassium hydroxid in bottom of container	80	20	0	0	0
Commercial wrappers, with 225 gr. soda lime in bottom of container	0	5	5	50	40

[1] Apples sealed in cans with arrangement for slow renewal of air.

The data in Table IX show a very striking relation between the prevention of the browning and gas absorbents. In these tests 95 per cent of the treated fruit was marketable, as compared to only 5 per cent of that of the controls. The figures also indicate a definite relation between the capacity of the various absorbents for taking up esters and the prevention of the disease. Paraffin, which according to Gildemeister and Hoffman (*11*) has an absorbing power of approximately one-half that of the other substances, showed the least prevention of browning. The poor showing made by the tallow-lard mixture was possibly due to the fact that it became rancid before the experiment was little more than started.

All the tests with gas absorbents, as well as those with air circulation, seem to indicate that internal browning is caused by the accumulation of certain materials in the nature of essential oils or other volatile substances.

A very perplexing question which then arises is that of the appearance of scald on the surface while internal browning develops in the flesh of the fruit. In an attempt to answer this question, the writer placed fruit under optimum conditions for the development of both diseases. That is, apples were placed in stagnant air at a temperature which favors the development of the disease. The results of these tests were very interesting. In every case it was found that the scald and browning developed almost simultaneously. The scald rapidly developed into what is termed "deep scald," while the browning diffused outward at a similar rate from the points of initial appearance about the vascular bundles. In many specimens where the diseases were retarded more on one side of the fruit than on the other, a very interesting comparison of their spread could be made. The generally observed appearance of scald on the surface without the internal browning and the reverse condition would then seem to indicate that these two regions of the fruit are most susceptible to the essential oils, or that these substances accumulate more pronouncedly in these than in any other region of the apple. The disease seemingly appears first in that region which is most

susceptible. In the Yellow Newtown, apparently, the region of greatest susceptibility is in the flesh, while in those varieties that scald readily, it is at the surface.

If internal browning and apple-scald are caused by the accumulation of essential oils, which can be removed by ventilation or by absorption, the question arises as to why the preventive action of ventilation and absorbents is less marked in the control of internal browning than in apple-scald. This difference in the effectiveness of the prevention is undoubtedly due to the fact that apple-scald is the result of the accumulation of deleterious substances on the surface of the fruit where the absorbent can be brought close to them. Internal browning, on the other hand, is caused by an accumulation of the deleterious substances deep in the tissues, from whence they can be removed only by the very slow process of reducing their concentration at the surface, thereby inducing them to diffuse outward.

INCREASE IN PERMEABILITY PRIOR TO THE APPEARANCE OF INTERNAL BROWNING

If internal browning is due to the action of some deleterious substance which tends to accumulate in the flesh of the apple under storage conditions, there should be some evidence of its action before the browning actually occurs. By this it is meant certain alterations will occur in the cells which will permit the browning to take place. Possibly the most important, as well as the most probable, change which could take place is that of altering the permeability.

The changes in permeability were determined by measuring the resistance offered by the tissue to the passage of an electric current. Electrodes for this purpose were patterned after those used by Small (*15*). The electrodes were mounted so that they stood 2½ mm. apart and in such a manner that they would be pressed ½ cm. into the tissue. The measurements were made by the Kohlrausch method. The fruit was cut as in making the observations upon the browning and the electrodes were then pressed into the various regions in which measurements were to be made. The readings were made by bringing the minimum point to the same position on the bridge each time. These readings, therefore, indicate only the relative resistance of the different regions in the fruits. (For the information of some readers it may not be amiss to state that a decrease in the resistance offered to the passage of an electric current is interpreted to mean an increase in the permeability of the cells.)

Since it was impossible to obtain the above apparatus until late in the season, the results which are recorded in Table X give only one stage in the permeability changes that occur during the course of an entire storage season. All the measurements, with the exception of those given under tests No. 4, 5, and 11, were made in tissue which showed no browning.

TABLE X.—*Permeability at the surface and in the region of browning*

[Expressed in ohms resistance]

Test No.	Fruit tested.	Majority of readings.		Extremes of variation.	
		In region of browning.	At the surface.	In region of browning.	At the surface.
1	Apples of lots 6 and 9, stored at 8.3° C.	1,600 to 1,800..	1,600 to 1,800..	1,500 to 2,000..	1,500 to 2,000.
2	Same as (1), but stored at 5° C...	2,300 to 2,500..	2,600 to 2,800..	2,000 to 2,600..	2,300 to 2,800.
3	Same as (1), but stored at 0° C. (These lots browned at 0° C.)	1,100 to 1,300..	2,300 to 2,500..	1,100 to 1,800..	2,200 to 2,800.
4	Same as (3), but reading taken in trace brown tissue.	900 to 1,200....	2,300 to 2,400..	800 to 1,300....	2,000 to 2,800.
5	Same as (3), but reading made in severely brown tissue.	600 to 900.....	2,200 to 2,400..	500 to 1,100....	1,900 to 2,600.
6	Fruit from black bags, very resistant to browning at 0° C.	2,200 to 2,600..	2,600 to 2,900..	2,000 to 2,900..	2,300 to 2,900.
7	Apples from same tree as bagged fruit, very susceptible to browning at 0° C.	1,000 to 1,200..	2,300 to 2,500..	1,000 to 1,600..	2,000 to 2,700.
8	Virginia apples, very resistant to browning at 0° C.	2,500 to 2,700..	2,600 to 2,800..	2,300 to 2,800..	2,500 to 2,800.
9	Santa Cruz Mountain apples, very resistant to browning at 0° C.	2,200 to 2,500..	2,500 to 2,800..	1,700 to 2,500..	2,300 to 2,800.
10	Lots 3, 5, and 8, very susceptible to browning at 0° C.	1,000 to 1,500..	2,400 to 2,800..	1,000 to 2,000	1,700 to 2,800.
11	Same as (10), but readings made in moderately browned tissue.	700 to 900.....	2,300 to 2,600..	500 to 1,100....	1,600 to 2,800.

The figures of Table X show very definitely that there is a change in the permeability. At 8.3° C., where browning does not develop, there was an increase in permeability. These apples, however, were rapidly approaching storage breakdown, due to overripening at this relatively high temperature. The permeability had not increased in the apples stored at 5° where the ripening process was much slower and where the fruit remained free from browning. This was also true of the fruit at 0° which was resistant to the browning. In the fruit stored at 0° that which was susceptible to the browning, there was a greater increase in permeability in the interior of the specimens than occurred in the fruit stored at 8.3°. The fruit at 0°, nevertheless, was not approaching storage breakdown but incipient browning. In the specimens showing browning the permeability continued to increase with the advance in the severity of the disease. Therefore these data seem to indicate that, just prior to and accompanying the end of the storage life of these apples, there is a very marked increase in permeability, regardless of whether deterioration is brought about by storage breakdown or by internal browning.

INCREASE IN PERMEABILITY DUE TO ESSENTIAL OILS

If internal browning be due to the accumulation of essential oils or similar deleterious substances which change the permeability, these oils should also increase the permeability when applied to the surface of the cut fruit. In order to test this property of these substances, several essential oils in great dilution were applied to the fruit about the electrodes of the conductivity apparatus. After the initial resistance was taken a drop of the solution was applied around the electrodes. Then the reading of the resistance was made every 5 minutes for a period of 20 minutes. The results of these tests were recorded in Table XI.

TABLE XI.—*Effect of essential oils upon the permeability of apple tissue*

[Expressed in ohms resistance]

Treatment.	The initial resistance.	Resistance at various intervals after the application of the essential oils.			
		After 5 minutes.	After 10 minutes.	After 15 minutes.	After 20 minutes.
Control, no treatment (current on continuously)	2,900	2,800	2,800	2,400	2,200
One drop of 0.1 per cent solution of amyl acetate	2,800	1,600	800	550	350
One drop of saturated solution of amyl valeriate	2,900	1,600	1,000	800	600
One drop of 1 per cent solution of acetaldehyde	2,900	1,600	1,000	750	500
One drop of 0.001 per cent solution of amyl acetate	2,900	2,000	1,100	900	600
One drop of 0.001 per cent solution of acetaldehyde	2,700	2,100	1,200	900	800
Water about the electrodes	2,800	2,400	2,200	2,100	2,000

The figures in Table XI show conclusively that essential oils increase the permeability when brought in contact with fruit tissues. These data indicate also that only a very small accumulation of essential oils might be sufficient to increase the permeability of apple cells, allowing the oxidase and substrates to come in contact, thus resulting in the browning. This is especially true when the greatest dilutions of the substances used in these tests are compared with the normal essential oil content of some apples as given by Power and Chesnut (*14*).

GENERAL DISCUSSION

In view of the relation of the browning with lower mean temperatures, it seems possible that the more severe browning of the mature fruit was due to exposure to the lower temperature which prevailed during the latter part of the harvesting season. The fruit of the second picking was exposed to a mean temperature of about 2.5° C. below the mean temperature of the growing season for three weeks after the fruit of the first picking was harvested. The fruit picked November 22 was exposed for six to eight weeks to the influence of a mean daily temperature of 2.5° to 8° below that prevailing at the time and before the first picking was made. As a whole, these data point to the possibility that the low temperature favors those conditions within the fruit which are necessary for the development of browning. This weakness in the fruit, if it can be considered as such, may be due to an abnormal development of the protoplasmic structure of the apples or to an accumulation of some deleterious substance which brings about a more rapid cessation in the normal functioning of these structures in storage. This seems probable since there was no appreciable difference between the resistant fruit and the fruit very susceptible to browning in constituents such as sugars, acid, and the P_H value of the expressed juice, which, it is generally believed, might influence a reaction of this sort through their effect upon the equilibria within the cells.

The accumulation of essential oils or similar deleterious substances also seems to be rather closely linked with the weakness in these apples that shows up in storage. This is indicated by the great reduction in the amount of browning that is brought about through the employment of air circulation or the impregnation of the wrappers with good absorbents for these substances. It has been further demonstrated that the permeability of the cells, which is the most probable change that might precede this browning or similar reactions, is increased very rapidly by essential oils when applied even in great dilution to the apple tissue. It was also found that there was an increase in permeability prior to and accompanying the death of the cells in the apple regardless of whether death was due to the usual type of storage breakdown (which is the result of overripening) or to internal browning.

The data obtained upon the relationship of temperature and the accumulation of essential oils or similar volatile substances to the browning, although not conclusive, point to several possibilities concerning the cause of this disease. When these apples are grown at a mean temperature as low as that of the growing season of the Pajaro Valley, they may fail to develop normally, hence when they are placed in storage the flesh of the fruit exhibits a susceptibility to injury through the action of the volatile emanation of the apple. This is indicated by the behavior of the fruit from different regions as well as by that from under the tent and in the black bags. This lower temperature may not only affect the development of the fruit but also apparently influence the production or accumulation of the volatile substances which are immediately responsible for the browning. This becomes apparent when the great difference in the amount of browning which developed at the several storage temperatures is taken into account. Seemingly there is a greater production of these substances at the lower temperatures, or otherwise they must accumulate more rapidly in those regions of the torus that are first to show the browning.

The reduction in the development of the browning by the use of gas absorbents also indicates that these volatile substances are present in injurious amounts at the lower temperatures under the ordinary conditions of storage. The more rapid accumulations of the deleterious substances may seem the more probable way of accounting for the injurious amount of these substances when the decrease in their volatility and the decrease in the permeability of the tissue at the lower temperatures is considered. However it is likely that there is also a greater production of these substances under the somewhat abnormal conditions of the lower temperatures of storage.

The nature of the process which results in the browning becomes of interest in connection with the preceding possibilities as to the cause of this trouble. Plausible explanations of this process could possibly be ascribed to an increase in the permeability of the protoplasm which permits the enzyms and their substrates to mix, or to the inactivation of some inhibiting substance. These changes might be brought about by the accumulation of certain substances such as the essential oils which are produced by the apple in storage and which apparently have a toxic effect upon the protoplasm of the cells.

In the normal cells the enzyms are prevented from acting upon their substrates by inhibitors or through lack of contact due to the possibly impermeable nature of the phase surface of the protoplasm. When the phase arrangements in the protoplasm, however, are acted upon

by toxins, these substances are no longer prevented from coming into contact. Similarly, the toxins may act upon the inhibitors to inactivate them. As a result of this liberation, the tannins of the apple cells may be oxidized to a brown by the oxidase which is also present in the mature fruit. It has been indicated by Bartholomew (*3*) that changes similar to these precede the blackening of the tissue in blackheart of potatoes. This explanation of coloration based upon a change in the permeability is also supported by the fact that before browning occurs there is a great increase in permeability of the cells as indicated by the conductivity measurements.

SUMMARY

(1) Internal browning is a nonparasitic disease of the large isodiametric cells of the flesh of the fruit.

(2) All Yellow Newtown apples, regardless of where grown, may in some years be susceptible to internal browning. This variety when grown under conditions prevailing in the Pajaro Valley has proved to be much more susceptible to this disease than when grown in other fruit regions.

(3) The later the fruit was picked, the greater the amount of browning which occurred in storage.

(4) No browning occurred even after four to six months storage in any of the fruit stored at 8.3° C. or above.

(5) The browning at the end of six months' storage at 5° C. was very limited and mild, and did not detract from the commercial value of the fruit.

(6) At 2.2° C. approximately 70 per cent of the apples showed browning by April 1, figures for each of the three seasons show. During the season 1920–21 which was average with regard to browning, only 50 per cent of the fruit stored at this temperature was marketable on April 1, 1921.

(7) At 0° C. practically all the fruit showed browning before April 1, each season. Only 20 per cent of the fruit stored at this temperature was marketable on April 1, 1921.

(8) A lowering by about 3° C. of the mean orchard temperature during the growing season, by tenting or shading a tree, greatly increased the susceptibility of the fruit to browning. After 4½ months' storage at 0° the fruit of the tented trees showed 25 per cent less of normal specimens than that of adjacent trees which were naturally exposed.

(9) An increase of 4° C. in the mean orchard temperature, by bagging individual apples in black cloth during the growing months, markedly increased the resistance of the fruit to this disease. The bagged apples showed 66 per cent more of normal specimens after 4½ months' storage at 0° than the naturally exposed fruit of the same trees.

(10) The browning was greatly reduced by ventilating the fruit.

(11) The browning was also reduced by impregnating the wrappers with oils and waxes which were good absorbents of essential oils.

(12) By measuring the electrical resistance of the apple tissue, it was found that there was an increase in permeability prior to the end of the storage life of the apple, regardless of whether death was due to the usual storage breakdown or to internal browning.

(13) It was demonstrated that essential oils when applied to the apple tissue even in great dilution rapidly increase its permeability.

(14) The data indicate that internal browning is due to the accumulation of essential oils or similar deleterious substances which are produced by the apples in storage. This shows that internal browning and apple-scald are quite closely related with respect to cause.

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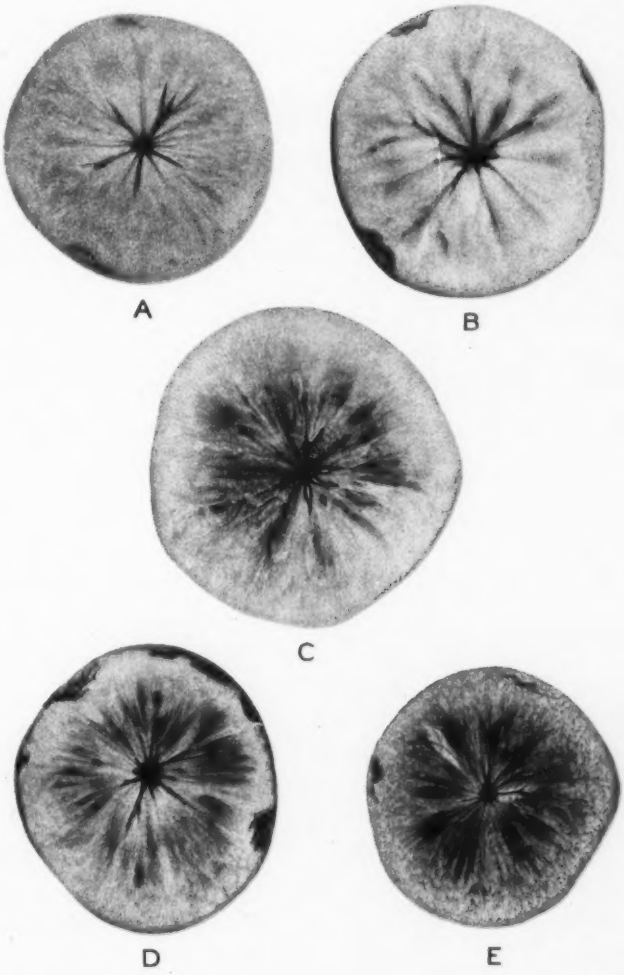
PLATE 1

A.—A normal apple.
B.—Average condition of trace browning.
C.—Average condition of slight browning.
D.—Average condition of moderate browning.
E.—Average condition of severe browning.


A
B
C
D
E

ON THE USE OF CALCIUM CARBONATE IN NITROGEN FIXATION EXPERIMENTS[1]

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In reviewing the literature on nitrogen fixation by soil bacteria one is impressed with the great variety of media that have been employed by different investigators. Many of these media, while generally satisfactory, have not proved entirely so when employed by other investigators with slightly different environmental conditions.

It is not the purpose of this paper to enter into any discussion of the relative merits of different media, but rather to call attention to a frequent fundamental difference and its possible bearing upon the success attending their use. This difference is the presence or absence of calcium carbonate.

Winogradsky (*9*)[2] in his original experiments on nitrogen fixation by anaerobic bacteria used a dilute solution of the various salts necessary to furnish the elements essential to growth. To this was added a simple sugar as a source of energy and an excess of calcium carbonate to neutralize the acids formed from the sugar. Winogradsky's medium has been almost universally adopted for anaerobic nitrogen-fixing experiments.

Beijerinck (*2*), studying the aerobic Azotobacter group of nitrogen-fixing bacteria, found that a 0.02 per cent solution of K_2HPO_4 in "Leitungswasser," to which was added a source of energy, furnished the necessary conditions for good growth of these organisms. Either the water or the inoculum must have furnished the other essential elements in sufficient quantity. The reaction of this medium was unaltered, the statement being made that—

> Die Nährlösung reagiert durch das K_2HPO_4 schwach alkalisch

and that—

> Die Alkalisch Reacktion ist für den versuch günstig.

Beijerinck preferred mannite or a salt of propionic acid as a source of energy because—

> Mannit kann nur schwierig und langsam, Propionate durchaus nicht der Buttersäuregärung anheimfallen.

Beijerinck further states that—

> Die Produkte die Oxydation sind Kohlensäure und Wasser.

However, he realized that in impure cultures from soil, organic acids might be formed. Beijerinck failed to secure appreciable fixation of nitrogen by pure cultures.

Lipman (*3*) began a study of the Azotobacter group of nitrogen-fixing organisms shortly after Beijerinck. He demonstrated that Beijerinck's failure to secure fixation in pure cultures was due to the unfavorable re-

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[2] Reference is made by number (italic) to "Literature cited," pp. 189-190.

action of his media. The media adopted by Lipman was composed of tap water 1,000 cc., mannite 15 gm., K_2HPO_4 0.5 gm., $Mg.SO_4$ 0.2 gm., a drop of 10 per cent solution of ferric chlorid, and enough sodium hydroxid to make the solution slightly alkaline to phenolphthalein. Lipman showed that within certain limits the quantity of nitrogen fixed was proportional to the quantity of sodium hydroxid added. He further demonstrated that the addition of $CaCO_3$, even to the medium made alkaline to phenolphthalein with sodium hydroxid, rendered it more favorable for nitrogen fixation. With regard to the influence of calcium carbonate Lipman (*4*) says:

> It is clear therefore, that the presence of calcium carbonate stimulated growth either directly by furnishing calcium, or indirectly by making available more phosphorus, sulphur, and magnesium.

However, Lipman apparently lost sight of the value of calcium carbonate, for he did not recommend its use in his laboratory guide (*5*).

Ashby (*1*), apparently following the lead of Lipman, proposed that the acidity arising from the phosphate be neutralized with sodium hydroxid and in addition an excess of calcium carbonate be added. The medium proposed by Ashby has been more widely used than any other. It has the following composition: Distilled water 1,000 cc., mannite 12 or 20 gm., $Mg.SO_4$ 0.2 gm., KH_2PO_4 0.2 gm., NaCl 0.2 gm., $CaSO_4$ 0.1 gm., and 0.5 gm. of $CaCO_3$ to each culture of 75 or 100 c. c. The phosphate is dissolved separately in a little water and made neutral to phenolphthalein with sodium hydroxid. Ashby found that the presence of calcium carbonate favored nitrogen fixation and that Azotobacter would sometimes develop in the presence of calcium carbonate but would not form a film if the carbonate were left out. Ashby also found that magnesium carbonate was even more efficacious than calcium carbonate, thus showing that calcium was not the essential constituent. Other investigators have since shown that other basic compounds can be substituted for calcium carbonate.

In addition to the three types of media just mentioned Löhnis and his students have made rather extensive use of soil extract to which was added K_2HPO_4 and mannite or some other simple organic source of energy. In comparing the fixation of nitrogen in a medium of this type with and without the addition of calcium carbonate Löhnis and Pillai (*7*) found, as a rule, slightly greater fixation where the carbonate was added. However, Löhnis failed to adopt the use of calcium carbonate generally in his work, or to recommend its use in his laboratory guide (*6*).

It remained for Stoklasa (*8*) to produce the necessary evidence for a correct understanding of the function of calcium carbonate in nitrogen-fixation experiments by demonstrating quantitatively the formation of organic acids in cultures of Azotobacter. A survey of the accumulated literature on the subject will show, however, that many investigators failed to realize the significance of Stoklasa's results.

Practically all investigators agree that a neutral or alkaline reaction is desirable, if not essential, for the best development of nitrogen-fixing organisms. In most work some effort is made to adjust the medium to an alkaline reaction before inoculating, but in many cases no effort is made to maintain such a reaction. Even the influence of the inoculum upon the initial reaction has usually not been taken into consideration.

So far as the writer is aware no one has ever reported a detrimental effect upon nitrogen fixation from the presence of calcium carbonate in

the medium, even when present in large excess. This is an important consideration, however, since if it has no toxic effect upon the organisms it may be added in excess of initial requirements and thereby tend to maintain a favorable reaction throughout the experiment.

There are a number of isolated experiments such as those cited above showing the effect of calcium carbonate upon the growth of nitrogen-fixing organisms. In the course of some experiments, conducted by the writer, in which the relative growth of Azotobacter from a large number of different soils was compared there was an opportunity to observe the effects of $CaCO_3$ on nitrogen fixation.

METHODS

The medium employed had the following composition: Mannite 20 gm., K_2HPO_4 0.2 gm., $MgSO_4$ 0.2 gm., NaCl 0.5 gm., $FeCl_3$ trace, and water 1,000 cc. Two-hundredths gm. of $CaCl_2$ was sometimes added, although in most cases because of the high calcium content of local soils the $CaCl_2$ is not essential and was without effect. In those tests to which no $CaCO_3$ was added the medium was always rendered slightly alkaline to phenolphthalein with sodium hydroxid. When $CaCO_3$ was to be added the medium was sometimes first rendered slightly alkaline to phenolphthalein and at other times the reaction was unaltered prior to the addition of the $CaCO_3$. Fifty cc. of the medium were placed in 300-cc. Erlenmeyer flasks, and the $CaCO_3$ was added in the form of sterile powder just prior to inoculation. No superiority is claimed for this medium over a score of others that might have been used. Obviously a medium with as variable composition as that containing tap water or soil extract would be unsuited for comparative work that must extend over a long period of time.

Samples were always set up in duplicate and total nitrogen determinations made on the whole sample. Total nitrogen determinations were also made in duplicate upon the inoculum. The inoculum consisted of 10 cc. of the supernatant suspension prepared by shaking one part of soil (50 to 100 gm.) with two parts of water and allowing to settle long enough for the larger particles to sink to the bottom. It is believed that such an inoculum is more representative of a mass of soil than 5 gm. of soil, and at the same time the quantity of solid material added is not sufficient to interfere in the least with total nitrogen determinations. Incubation was at room temperature for three weeks. In estimating the quantity of nitrogen fixed that present in the inoculum was deducted. Only the average of check determinations were recorded.

Frequent examinations of the cultures were made, both macroscopically and microscopically to ascertain whether Azotobacter were present. If Azotobacter make an appreciable growth it can usually be recognized by the appearance of the film. A microscopic examination of an unstained mount from such a film will reveal an unmistakable picture. A film is sometimes encountered which at certain stages in its development resembles quite closely an Azotobacter film, which, under the mircoscope, is found to be composed almost entirely of filimentous fungi, no organisms typical of Azotobacter being observed. In other instances nontypical films examined under the microscope would be found to be composed largely of fruiting fungi, the spores of which often closely resembled individual cells of Azotobacter.

If these examinations failed to reveal organisms morphologically similar to Azotobacter they were regarded as absent. Owing to the above-mentioned complex conditions it is quite possible that Azotobacter were sometines reported present when in reality they were absent and vice versa. The end to be gained did not seem to justify the large amount of time that would be necessary to isolate and identify Azotobacter from the various soils. It is believed that if Azotobacter are not present in a soil in sufficient numbers and vigor to develop unmistakable evidence of their presence by the methods just described, for practical purposes they may as well be absent.

RESULTS

Several hundred samples of soil from Kansas and other States have been examined by the methods described above. The following is a comparison of the average quantity of nitrogen fixed by 200 soils.

All samples	5.87 mgm.
Presence of $CaCO_3$	7.10 mgm.
Absence of $CaCO_3$	4.60 mgm.
Azotobacter film formed	7.70 mgm.
No Azotobacter film formed	4.10 mgm.

There were only two samples that failed to show some nitrogen fixation, and both of these were in media containing no $CaCO_3$.

When calcium carbonate was added to the medium an Azotobacter film was formed from 117 samples, or 58 per cent of the soils. The average quantity of nitrogen fixed in these was 8.1 mgm. The average quantity of nitrogen fixed in the 83 samples having no Azotobacter film was 5.7 mgm.

When no calcium carbonate was added to the medium an Azotobacter film was formed from 75 samples, or 38 per cent. These had fixed on the average 7.1 mgm. of nitrogen. One hundred and twenty-four samples, or 62 per cent, produced no Azotobacter film, and the average nitrogen fixed for these was 3.1 mgm.

Twenty-seven samples, or 14 per cent of all soils examined, fixed more nitrogen in the samples to which no $CaCO_3$ was added, while 173 samples, or 86 per cent, fixed larger quantities of nitrogen in those samples receiving an addition of $CaCO_3$. The microscope revealed Azotobacter in cultures from 130 samples, or 65 per cent of all. No Azotobacter were observed in cultures from 70 samples, or 35 per cent of all. Some nitrogen fixation took place in practically all samples inoculated regardless of the source of the soil.

There were 12 samples containing Azotobacter or organisms resembling Azotobacter that failed to form an Azotobacter film. The average nitrogen fixed by these 12 soils where $CaCO_3$ was added was 6.2 mgm. The average in the absence of $CaCO_3$ was 3.1 mgm. This is 0.5 mgm. higher than the average fixed by those giving no film when $CaCO_3$ was added and exactly the same as those giving no film in the absence of $CaCO_3$. It is highly probable, therefore, that some soils contain Azotobacter but are incapable of initiating the growth of an Azotabacter film in a mannite culture solution.

Practically all soils that failed to produce Azotobacter films formed more or less heavy films of fungi in the medium containing $CaCO_3$. As a rule, no such films were formed in the medium containing no $CaCO_3$. Whether or not these fungi are associated with the increased

nitrogen fixation under these conditions is not known. It is possible that the films of aerobic fungi were a factor in maintaining anaerobic conditions and thereby stimulated nitrogen fixation by anaerobic organisms. The fungi were usually slow to develop, indicating that their development depended upon some subsequent change, possibly the accumulation of nitrogen or of calcium salts of some organic acids. The number of samples that failed to develop fungi films were hardly sufficient to give a comparison of the quantity of nitrogen fixed in the presence and in the absence of a film. It is perhaps significant, however, that the average quantity of nitrogen fixed by the 7 samples which failed to grow films of fungi in the presence of $CaCO_3$ was only 2.6 mgm., compared with 6.0 mgm. for the 76 samples producing a film. This would indicate that the fungus growth is in some way associated with the fixation of nitrogen either as a factor or as a result.

It is evident from the preceding data that practically all soils will bring about the fixation of appreciable quantities of nitrogen under the conditions of these experiments. A large percentage of the soils examined however, failed to initiate the growth of Azotobacter. There are, therefore, other organisms which are capable of fixing appreciable quantities of nitrogen. Such organisms seem to be quite widely distributed in nature.

CONCLUSIONS

(1) The quantity of nitrogen fixed in the presence of Azotobacter is greater than when it fails to develop.

(2) The number of soils capable of initiating the growth of Azotobacter under the experimental conditions here described is greater by 20 per cent if $CaCO_3$ is added to the medium than if it is omitted.

(3) The quantity of nitrogen fixed in a medium containing $CaCO_3$ is, for practical purposes, always equal to and in most cases greater than when $CaCO_3$ is not present in the medium.

(4) The presence of $CaCO_3$ exerts a greater beneficial effect upon those organisms, other than Azotobacter, that bring about the fixation of nitrogen than upon Azotobacter itself.

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